NUTRITIONAL COMPOSITION AND SENSORY EVALUATION OF SPIRULINA (*Arthrospira platensis*) MILLET-BASED COMPOSITE FLOUR FOR CHILDREN OF 6 TO 59 MONTHS

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A DISSERTATION SUBMITTED TO THE DIRECTORATE OF RESEARCH AND GRADUATE TRAINING IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN FOOD TECHNOLOGY OF KYAMBOGO UNIVERSITY

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DECLARATION

I, Laker Scovia, confirm that this study is my original work and has never been submitted, in whole or in part, to any academic institution for qualification or award.

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APPROVAL

We hereby affirm that this project was carried out by the candidate under our supervision and is ready for submission with our approval.

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DEDICATION

I dedicate this work to my dear parents, Mr. Okidi Justine Law and Ms. Ayot Christine, in gratitude for their unwavering support throughout my academic journey.

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I thank the Almighty God for the gift of life, health, wisdom, and strength that enabled me to complete this research.

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LIST OF ACRONYMS AND ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
ANFs	Antinutritional Factors
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CIU-REC	Clark International University Research Ethics Committee
EU	European Union
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GRAS	Generally Recognized as Safe
MOH	Ministry of Health
PEM	Protein Energy Malnutrition
SPSS	Statistical Package for the Social Sciences
UBOS	Uganda Bureau of Statistics
UDHS	Uganda Demographic and Health Survey
UIRI	Uganda Industrial Research Institute
UNCST	Uganda National Council for Science and Technology
UNICEF	United Nations International Children’s Emergency Fund
UV-VIS	Ultraviolet–Visible Spectrophotometer
WFP	World Food Programme
WHO	World Health Organization

ABSTRACT

Millet is used as complementary food for children between the ages of 6 and 59 months. The presence of anti-nutritional factors in millet reduces nutrient bioavailability and makes millet-based diet fail to meet young children's nutrient requirement. This study evaluated the nutritional composition and sensory acceptability of spirulina (*Arthrospira platensis*) millet-based composite flour developed from *Ekaama, Odyera,* and *Ekwangapel* millet varieties for children under five years of age. A completely randomized factorial design was employed to examine proximate composition, mineral levels, anti-nutritional constituents, β-carotene concentration, and sensory performance of the formulated flours. Both flours from germinated and non-germinated millet were analyzed following standard AOAC protocols. Mineral determination was carried out using Atomic Absorption Spectrophotometry, while anti-nutritional factors and β-carotene were determined using UV–Visible spectrophotometric techniques. Different spirulina inclusion levels (0 to 10%) were developed using Nutri-Survey software. Porridges prepared from these blends were assessed by an untrained panel using a nine-point hedonic scale for colour, aroma, texture, taste, mouthfeel, and overall acceptability. Germination increased ($p < 0.05$) protein content by 23.1%**,** fat by 107.9%**,** calcium by 12.7%**,** iron by 70.2%**,** and zinc by 108.7%**.** It reduced moisture content by 13.1%**,** ash by 17.6%**,** fibre by 20.0%**,** carbohydrate by 1.1%**,** and magnesium by 9.1%**.** Among the varieties, *Odyera* indicated the highest protein concentration (9.97%), followed by *Ekaama* (9.59%) and *Ekwangapel* (9.44%). *Odyera* showed superior mineral levels across the analyzed varieties. Across eight millet types (*Ekwangapel, Ekaama, Emoro moru, Ayuku manyige, Odyera, Min bel, Luk caa,* and *Adyang adyang*), oxalates by 82.0%, phytates by 32.8%, and tannins by 60.1%. Incorporation of 10% spirulina into *Odyera*-based flour yielded the greatest protein (15.76%), iron (16.73 mg/100 g), and β-carotene (353.24 mg/kg) concentrations. Sensory analysis indicated that porridge from germinated *Ekaama* with 2% spirulina was most preferred (score 7.57), followed by *Odyera* (7.23) and *Ekwangapel* (6.67) at the same level. Higher spirulina enrichment generally reduced consumer preference, mainly due to green coloration and flavor alterations. Nonetheless, germination coupled with moderate spirulina addition (2%) notably improved both nutritional quality and sensory acceptance of millet-based complementary foods prepared from *Ekaama, Odyera,* and *Ekwangapel*. These findings demonstrate the potential of such formulations to provide nutrient-dense and culturally suitable foods to alleviate Protein Energy Malnutrition among children.

Keywords: Millet, Spirulina, Germination, Antinutrients, Composite flour, Protein, Iron, β-carotene, Complementary feeding, Sensory quality.

CHAPTER ONE: INTRODUCTION

1.1 Background

Malnutrition among children aged 6 to 59 months remains a major global health concern. Worldwide, about 149 million children are stunted, 45.4 million suffer from wasting, and roughly 38.9 million are overweight (UNICEF, WHO, and World Bank Group, 2021). Sub-Saharan Africa bears the highest impact, accounting for 56.8% of stunted, 2.2% of wasted, and 6.6% of overweight cases (UNICEF and WHO, 2023). In Uganda, Protein Energy Malnutrition (PEM) contributes to nearly 45% of mortality in children aged 6 to 59 months. According to national surveys, 14.3% of Ugandan children are affected by PEM, 26% experience stunting, and 10% are underweight (UDHS, 2022; UBOS and ICF, 2016). Stunting prevalence varies geographically, being highest in Eastern Uganda, followed by the Northern, Central, and Western regions (UDHS, 2022). PEM compromises normal growth, slows brain development, weakens immunity, and raises infection risks (Govender, Rangiah, Kaswa, Nzaumvila, 2021).

Although Uganda has adopted several nutrition strategies such as food fortification, supplementation, dietary diversification, and biofortification most focus primarily on micronutrients rather than addressing protein energy deficiencies, thus limiting their effect on protein adequacy (Msungu, Mushongi, Venkataramana and Mbega, 2022; MOH, 2020; Schuftan, Ramalingaswami and Levinson, 1998). Millet (*Eleusine coracana*), a staple crop across Africa and Asia (Kheya, Talukder, Datta, Yeasmin, Rashid, Hasan, Anwar and Islam, 2023), serves as complementary food for Ugandan children aged 6 to 59 months, typically consumed as porridge or pancakes (Ugada, Okidi and Ongeng, 2023). It supplies carbohydrates, protein, fiber, and essential micronutrients but contains compounds such as tannins, oxalates, and phytates that inhibit nutrient absorption (Samtiya, Aluko and Dhewa 2020). Protein levels vary across millet species, including *Eleusine coracana*, *Pennisetum glaucum*, *Setaria italica*, *Echinochloa esculenta*, and *Panicum miliaceum* (Sachdev, Goomer, Singh, Pathak, Aggarwal and Chowhan, 2023). Germination reduces antinutritional factors and enhances nutrient digestibility. However, millet alone remains insufficent to meet children's protein and energy requirement, necessitating fortification with high-quality protein sources (Abioye, Babarinde, Ogunlakin, Adejuyitan, Olatunde, and Abioye, 2022).

Spirulina (*Arthrospira platensis*), a filamentous cyanobacterium provides readily digestible protein and micronutrients, enhancing nutrient bioavailability. It contains 60 to 70% high-quality protein with all essential amino acids (lysine, methionine, threonine, leucine, isoleucine, valine, phenylalanine, tryptophan, and histidine) (Tadros, 1990) and is rich in iron (580–1800 mg/kg, mainly in ferric pyrophosphate and chelated forms), zinc, calcium, magnesium, β-carotene, vitamins, and beneficial lipids, with iron being highly bioavailable compared to cereals (AlFadhly, Alhelfi, Altemimi, Verma and Cacciola, 2022). When sourced from certified producers, spirulina is considered safe and nutritionally superior to most plant-based foods (Othoo, Ochola, Kuria and Kimiywe, 2021). Integrating spirulina into flour from germinated millet enhances protein levels, mineral absorption, and antioxidant properties. While earlier enrichment studies have emphasized legume or micronutrient fortification, limited work has explored spirulina as a protein enhancer in millet-based complementary foods. Therefore, this study aimed to formulate and evaluate spirulina-enriched millet composite flours to enhance both the nutritional and sensory quality of foods designed for children aged 6 to 59 months.

1.2 Statement of the problem

Millet is naturally bulky due to its high fibre and carbohydrate composition, which increases porridge thickness and limits energy concentration. *Eleusine coracana* contains several anti-nutritional constituents (Abioye *et al*., 2022). These compounds interact with essential nutrients such as iron, zinc, calcium, and proteins to form insoluble complexes, thereby lowering their bioavailability (Samtiya *et al*., 2021). As a result, millet alone may not sufficiently provide the protein and energy required for children's optimal growth, since children have small limited capacities. Incorporating Spirulina (*Arthrospira platensis*) can help improve the nutritional profile of millet-based complementary foods. It is a nutrient rich microalgae containing 60 to 70% protein, essential amino acids, vital minerals, and β-carotene (AlFadhly *et al*., 2022; Othoo *et al*., 2021). Its inclusion has shown promising outcomes in enhancing the nutritional value of other cereals like corn (Othoo *et al*., 2021). However, limited literature exist in incorporating millet flour with spirulina as well as the effect of germination on the nutritional and functional characteristics.

1.3 Objectives

1.3.1 General objective

To evaluate nutritional composition and sensory evaluation of spirulina (*Arthrospira platensis*) millet-based composite flour for children of 6 to 59 months.

1.3.2 Specific objectives

1. To determine the proximate composition (moisture content, ash content, fiber content and protein content) of flour from non-germinated and germinated millet of eight local millet

varieties (*Ekwangapel, Ekaama, Emoro moru, Ayuku manyige, Odyera, Min bel, Luk caa* and *Adyang adyang*).

2. To quantify the concentration of minerals (zinc, calcium, magnesium and iron) of flour from non-germinated and germinated millet of eight local millet varieties.
3. To quantify the concentration of anti-nutritional factors (oxalates, tannins and phytates) of flour from non-germinated and germinated millet of eight local millet varieties.
4. To assess the consumer acceptability of the porridge prepared from spirulina millet-based composite flour of three selected superior local millet varieties (*Odyera, Ekaama* and *Ekwangapel*).
5. To determine the concentration of protein, iron and β-carotene in spirulina millet based composite flour of three selected superior local millet varieties (*Odyera, Ekaama* and *Ekwangapel*).

1.4 Hypotheses

1. There is no difference in the proximate composition (moisture content, ash content, fiber content and protein content) of flour from non-germinated and germinated millet of eight local millet varieties (*Ekwangapel, Ekaama, Emoro moru, Ayuku manyige, Odyera, Min bel, Luk caa* and *Adyang adyang*).
2. There is no difference in the concentration of minerals (zinc, calcium, magnesium and iron) of flour from non-germinated and germinated millet of eight local millet varieties.
3. There is no difference in the concentration of anti-nutritional factors (oxalates, tannins and Phytates) of flour from non-germinated and germinated millet of eight local millet varieties.

4. There is no difference in the consumer acceptability of the porridge prepared from spirulina millet-based composite flour of three selected superior local millet varieties *(Odyera, Ekaama* and *Ekwangapel*).
5. There is no difference in the concentration of protein, iron and β-carotene in spirulina millet based composite flour of three selected superior local millet varieties (*Odyera, Ekaama* and *Ekwangapel*).

1.5 Justification

This research aligns with Uganda's Nutrition Action Plan and National Development Plan IV (NDP IV), both of which prioritize dietary diversification, promotion of indigenous foods, and reduction of child malnutrition. It also contributes to Sustainable Development Goals 2 (Zero Hunger) and 3 (Good Health and Well-being) by producing a nutrient-rich, affordable, and culturally acceptable complementary food. The study's primary beneficiaries are children aged 6 to 59 months and their caregivers. Children gain improved access to nutrient-dense complementary foods, reducing malnutrition risk. Caregivers acquire knowledge and practical skills for preparing spirulina-fortified millet porridge, supporting healthy growth and development. Indirect beneficiaries include nutritionists, health practitioners, policymakers, and small-scale food processors. Nutritionists and health workers benefit from evidence-based data to guide interventions and nutrition education, while policymakers can strengthen child nutrition policies and fortification programs. Small-scale processors gain opportunities for product development, value addition, and income generation through local production of fortified complementary foods.

CHAPTER TWO: LITERATURE REVIEW

2.1 Malnutrition as a global public health challenge

Malnutrition occurs when the body fails to obtain adequate or balanced nutrients and is broadly categorized into undernutrition and micronutrient deficiencies, manifested through stunting, wasting, or being underweight (Scrinis, 2020). It remains a pressing global health issue that affects populations across all age groups. The COVID-19 pandemic further intensified this challenge, with approximately 149 million children reported as stunted, 45.4 million as wasted, and 38.9 million as overweight worldwide (UNICEF and WHO, 2023). This deterioration slowed the gains made in combating child malnutrition and hindered progress toward Sustainable Development Goal 3, which seeks to ensure good health and well-being by 2030 (FAO, IFAD, UNICEF, WFP, and WHO, 2020). Presently, about 54% of stunted children under five live in Asia, while nearly 40% are in Africa (WHO, 2021). In Sub-Saharan Africa alone, an estimated 56.8% of children are stunted, 2.2% are wasted, and 6.6% are overweight (UNICEF and WHO, 2023).

East Africa records one of the highest stunting rates globally among children under five (Neufeld, Beal, Larson and Cattaneo, 2020; Suberu, Maalim, Akinola and Faseun, 2020). Uganda continues to face serious nutritional challenges, ranking among the countries with the greatest prevalence of child undernutrition in the region. Data from the Uganda Demographic and Health Survey (UDHS, 2022) show that 26% of Ugandan children under five are stunted, 10% underweight, 3% overweight, and 3% wasted. Comparative figures indicate that Kenya reports 18% stunting, 10% underweight, 3% overweight, and 5% wasting (KDHS, 2022);

Tanzania records 30% stunting, 8% underweight, 1% overweight, and 5% wasting (TDHS-MIS, 2022); while Rwanda shows 33% stunting, 8% underweight, 6% overweight, and 1% wasting (RDHS, 2019–2020). These statistics demonstrate the persistent and region-wide challenge of child malnutrition across East Africa, emphasizing the urgency for locally tailored strategies to enhance child nutrition and health outcomes.

2.1.1 Macronutrient malnutrition in Uganda

Protein Energy Malnutrition (PEM), a key type of macronutrient deficiency, continues to be a major nutritional concern among Ugandan children aged 6 to 59 months (Adebisi, Ibrahim, Lucero-Prisno, Ekpenyong, Micheal, Chinemelum and Sina-Odunsi, 2019). About 14.3% of children are affected, with 26% stunted, 10% underweight, 3% overweight, and 3% wasted (UNICEF and WHO, 2023; UDHS, 2022; UBOS and ICF, 2016). Regional data from the Uganda Demographic and Health Survey (UDHS, 2022) indicate that stunting is highest in the Eastern region (26.8%), followed by the Western (21.0%), Northern (20.5%), and Central (20.0%) regions. Wasting follows a similar trend, peaking in the Eastern region (4.7%) and declining across the Northern (3.0%), Western (2.1%), and Central (1.9%) regions. Underweight prevalence is also greatest in the Eastern region (13.4%), compared with 9.6% in the Western, 9.4% in the Northern, and 7.6% in the Central region. Overweight cases are most common in the Eastern region (8.4%), followed by Central (4.8%), Western (4.4%), and Northern (1.3%) regions (Table 1).

Malnutrition shows clear regional variation in Uganda. Kampala has relatively lower rates, while Karamoja experiences some of the highest levels, with around 45% of children under five years affected, compared with just 5.7% in Kampala (WHO, 2023). Despite natural resources such as livestock and minerals, Karamoja faces economic marginalization due to poor infrastructure, recurring droughts, insecurity, and limited access to markets, which reduce food security and household incomes (UNFPA, 2018). Key factors contributing to macronutrient malnutrition in Uganda include low dietary diversity, poverty, limited health knowledge, restricted access to healthcare, poor sanitation, and inadequate weaning practices (Kiran, Wakeel, Mahmood, Mubaraka, Hafsa and Haefele, 2022; Turyashemererwa, Kikafunda and Agaba, 2009). Consequences for children include stunted growth, delayed cognitive development, weakened immunity, and increased susceptibility to diseases (Kiran *et al*., 2022; Soliman, De Sanctis, Alaaraj, Ahmed, Alyafei, Hamed and Soliman, 2021).

Table 1: Nutritional status of infants under five years in Uganda

Nutrition status (%)				
Region	**Stunting**	**Wasting**	**Underweight**	**Overweight**
Central	20.05	1.90	7.60	4.75
Eastern	26.84	4.74	13.40	8.44
Northern	20.53	3.00	9.40	1.27
Western	2.10	2.10	9.63	4.38

Source: (UDHS, 2022).

2.1.2 Micronutrient malnutrition in Uganda

Micronutrient deficiencies, which arise when essential vitamins and minerals are not adequately consumed or absorbed, remain a major public health concern in Uganda, particularly among children under five (Adebisi *et al*.,2019). The most prevalent deficiencies involve vitamin A, iron, iodine, and zinc (Nankumbi, Grant, Sibeko, Mercado, Kwikiriza, Heck and Cordeiro, 2023; Savarino, Corsello and Corsello, 2021). Vitamin A deficiency affects 8.3% of children in urban areas and 9.0% in rural settings, with higher rates in subregions such as Acholi (15.4%), Busoga (12.8%), and West Nile (11.2%) (UBOS and ICF, 2016). Anemia prevalence among children under five has increased from 50% to 53% (UBOS, 2016), with Gulu District reporting the highest rate in Northern Uganda at 46.6% (Ocan, Oyet, Webbo, Mwambi and Taremwa, 2018). In the Acholi subregion, anemia affects approximately 71% of young children, far exceeding the global average of 43% reported by WHO (2023).

Research by Nankinga, Olivia, Danstan Aguta and Catherine Kabahuma, (2019) shows high anemia prevalence across Uganda, including 66.2% in Northern Uganda, 52.4% in the Eastern region, 49.6% in the Western region, and 32.9% in the Southwestern region. Contributing factors include limited consumption of animal-source foods, scarce availability of fortified products, low dietary diversity, and nutrient losses during food processing. Methods such as milling, polishing, and extended cooking can reduce heat-sensitive vitamins like vitamin C and folate, as well as certain minerals, through oxidation, leaching, or removal of nutrient-rich layers (Lee, Choi, Jeong, Lee and Sung, 2018). To address these deficiencies, Uganda has implemented interventions including dietary diversification, food fortification, biofortification, and micronutrient supplementation to reduce malnutrition in children (Gohara-Beirigo, Matsudo, Cezare-Gomes, de Carvalho and Danesi, 2022; MOH, 2020).

2.2 Factors contributing to malnutrition in Uganda

Malnutrition in Uganda is influenced by a mix of social, economic, environmental, and health-related factors (Adebisi *et al*., 2019). A significant portion of the population lives below the national poverty line, which was estimated at 20.3% in 2019/20 and rises to nearly 30% under a revised threshold. This economic hardship limits access to sufficient and nutritious food, contributing to high malnutrition rates (World Bank, 2022). Many Ugandans rely on subsistence farming, which is highly vulnerable to climate variability and inefficient agricultural practices (Babyenda, Kabubo-Mariara and Odhiambo, 2023). Extreme weather events such as droughts, floods, and unpredictable rainfall patterns adversely affect crop production and food availability (Kogo, Kumar, and Koech, 2021). Limited access to fertile land due to population pressure and land tenure restrictions further constrains agricultural output (Maja and Ayano, 2021).

Other major contributors to malnutrition include high disease burden, poor sanitation, and limited access to healthcare services (Adebisi *et al*., 2019). Inadequate knowledge of proper nutrition and dietary practices often results in micronutrient deficiencies (Weerasekara, Withanachchi, Ginigaddara and Ploeger, 2020). Cultural beliefs and food taboos can restrict consumption of nutrient-rich foods by women and children, worsening malnutrition (Lekey, Masumo, Jumbe, Ezekiel, Daudi, Mchome, Leyna, 2024). For example, in some Ugandan communities, pregnant women avoid eggs, certain meats such as goat, and fish, while young children may be restricted from consuming eggs or milk during early development (Acire, Bagonza and Opiri, 2023). Similar restrictions exist in West African countries; for instance, in Benin and Togo, pregnant women traditionally avoid snails or cane rat meat (Lokossou, Tambe, Azandjèmè and Mbhenyane, 2021). Additionally, political instability and conflicts, particularly in Northern Uganda, have disrupted farming, limited market access, and reduced food availability, thereby heightening food insecurity and vulnerability to malnutrition (Abdullahi, Kalengyo and Warsame, 2024; Kibaate, 2009).

2.3 Strategies to combat malnutrition

The Ministry of Health in Uganda has implemented various strategies to combat malnutrition, including dietary diversification, food fortification, biofortification, and food supplementation (MOH, 2020; UNAP, 2021).

2.3.1 Food fortification

Food fortification involves deliberately adding essential vitamins and minerals to staple foods to improve nutrition and prevent deficiencies (WHO, 2006). In Uganda, the Ministry of Health recognizes fortification as a key strategy to address malnutrition (MOH, 2020). Examples include enriching maize flour with iron and folic acid to combat anemia and reduce the risk of neural tube defects. Research shows that such fortification efforts have increased folate and vitamin A levels and lowered anemia prevalence among children (Keats, Neufeld, Garrett, Mbuya and Bhutta, 2019). Similarly, iodized salt supports thyroid health and cognitive development, helping prevent iodine deficiency disorders (WHO, 2014). The use of vegetable oil fortified with vitamin A has also helped decrease vitamin A deficiency rates (USAID, 2022). The Ministry collaborates closely with food manufacturers to ensure compliance with national standards and monitors fortified products to maintain their nutritional quality and public health impact (MOH, 2020; MOH, 2011).

2.3.2 Bio-fortification

Biofortification is the process of enhancing the nutrient content of staple crops through plant breeding, agronomic techniques, or genetic modification, providing a sustainable approach to combat micronutrient deficiencies (WHO, 2006). In Uganda, orange-fleshed sweet potato varieties, such as NASPOT 8 and 13, which are high in pro-vitamin A, are widely grown in districts including Masindi, Lira, and Hoima, helping to improve vitamin A status among children (Nankumbi *et al.*, 2023). Iron-rich bean varieties, particularly NABE 1 and 2, primarily cultivated in central and western regions, support better iron intake and cognitive function. Pro-vitamin A banana varieties, such as M9 and M17, grown in Mukono and Masaka, also contribute to reducing vitamin A deficiency (Mbabazi, Harding, Khanna, Namanya, Arinaitwe, Tushemereirwe and Paul, 2020). Studies indicate that consumption of these biofortified crops raises serum retinol and hemoglobin levels in both children and adults (Lee, 2022). The Ministry of Health, together with the National Agricultural Research Organisation (NARO) and HarvestPlus, actively promotes the production, dissemination, and adoption of these nutrient-dense crops among at-risk populations (MOH, 2020).

2.3.3 Supplementation

Supplementation refers to the administration of essential vitamins or minerals, either orally or via injections, to maintain adequate nutrient levels over a specific period (MOH, 2020). For instance, distributing vitamin A capsules to children aged 6 to 59 months strengthens immunity and lowers the risk of infection-related mortality (Abdi, 2021; MOH, 2020). Similarly, providing iron and folic acid tablets to pregnant women helps prevent anemia and improves health outcomes for both mothers and their children (Luwangula, McGough, Tetui, Wamani, Ssennono, Agabiirwe, Michaud-Létourneau, Tumwesigye, Baleeta and Rwegyema, 2022; USAID, 2022; MOH, 2020). Additionally, regular deworming programs for children help reduce parasitic infections, which contribute to malnutrition (MOH, 2020).

2.3.4 Dietary diversification

Dietary diversification emphasizes consuming a wide variety of foods from different groups to fulfill nutrient requirements and enhance overall diet quality (WHO, 2006). In Uganda, including at least four of the seven key food groups (cereals, roots and tubers, legumes and nuts, dairy products, flesh foods, eggs, and fruits and vegetables) rich in vitamin A helps prevent micronutrient deficiencies and supports healthy growth in children (WHO and UNICEF, 2008). School feeding programs in districts such as Kampala and Wakiso have increased meal satisfaction, improved attendance by up to 12%, and positively influenced academic performance (Isiko, 2022). The Ministry of Health, in collaboration with UNICEF and the World Food Programme (WFP), implements community-level initiatives and food assistance programs to promote dietary diversity, especially among children under five and vulnerable households (USAID, 2022; MOH, 2020).

2.4 Production and consumption of millet

Millet (*Eleusine coracana*) is a cereal widely cultivated across the world, particularly in Asia and Africa (Ramashia, Mashau, and Onipe, 2021). Global millet production exceeds 4.5 million tons annually, with Africa contributing approximately 2.5 million tons (Kavi Kishor, Anil Kumar, Naravula, Hima Kumari, Kummari, Guddimalli, Edupuganti, Karumanchi, Venkatachalam and Suravajhala, 2021). Sub-Saharan Africa accounts for about 55 to 60% of global millet output, including major producing countries such as Ethiopia, Kenya, Malawi, Nigeria, Sudan, Uganda, Zambia, and Zimbabwe (Ramashia, Mashau, and Onipe, 2021). In Uganda, millet production is estimated at around 276,928 metric tonnes (UBOS, 2020). While millet is grown throughout the country, production is concentrated in the Eastern, Northern, Western, and Central regions (UBOS, 2020; Kasule, Kakeeto, Tippe, Okinong, Aru, Wasswa, Oduori and Adikini, 2023), with the Eastern region alone producing 77,784 metric tonnes more than the combined total of the other three regions (UBOS, 2020). Between 2008 and 2009, Uganda's millet production and consumption were recorded at 276,928 and 104,402 metric tonnes, respectively (UBOS, 2020) (Table 2).

Table 2: Regional production and consumption of millet in Uganda

Region	Production	Consumption
Central	13,734	3,126
Eastern	104,838	39,123
Northern	78,572	35,775
Western	77,784	26,378
Uganda	276,928	104,402

Source: (UBOS, 2020)

In Uganda, the Ankole sub-region recorded the highest annual millet production in 2020 at 16,000 metric tonnes, followed by Acholi with 12,000 MT, West Nile with 1,100 MT, and Bunyoro with 580 MT, according to sub-regional data (UBOS, 2020). Despite these figures, average on-farm yields remain below 1 ton per hectare, indicating that national millet production is still relatively low. Several factors limit productivity, including finger millet blast disease, a fungal infection that damages crops; inadequate post-harvest handling leading to storage losses; drought stress reducing water availability; and poor soil fertility limiting nutrient uptake (Kasule *et al*., 2023). To boost production, millet cultivation is being extended into previously underutilized areas such as the Southern drylands and highlands, the mid-Northern and Northern regions, West Nile farmlands, the Western highlands, and Karamoja drylands. These areas are highly suitable for finger millet (*Eleusine coracana*) due to favorable soil characteristics, climate conditions, and agro-ecological suitability (Adikini, Roggers, Ojulong, Aita, Opie, Wandulu, Aru and Ugen, 2021; Owere, Tongoona, Derera and Wanyera, 2014).

2.4.1 Nutritional composition of millet

Millet is a hardy cereal crop known for its short growing cycle and strong tolerance to drought, making it an ideal crop for addressing malnutrition (Kadapa, Gunturi, Gundreddy, Kalwala and Mogallapu, 2023). When processed into flour, millet is used to prepare a variety of foods, including bread, pancakes, porridge, bushera, kwete, muffins, biscuits, and cakes (Kasule *et al*., 2023). It is considered a valuable source of functional foods due to its rich composition of carbohydrates, dietary fiber, proteins, fats, and energy, as summarized in Table 3 (Ramashia, Mashau and Onipe, 2021). Additionally, millet provides substantial amounts of essential minerals, including calcium, iron, magnesium, potassium, phosphorus, and zinc (Ramashia, Mashau and Onipe, 2023) (Table 3).

Table 3: Nutritional composition of millet grains

Contents	Fioxtail millet	Kodo millet	Barnyard millet	Pearl millet
Proximate composition (g/100g)				
Moisture	11.2	12.8	11.9	12.4
Fat	2.38-4.3	1.3	2.2	4.8-5.0
Ash	0.47-3.3	2.6	4.4	2.2-2.3
Protein	11.50-12.3	9.8	6.2	11.6-11.8
Carbohydrates	60.9-75.2	65.9-66.6	65.5	67-67.5
Dietary fiber	2.5-8.5	2.47	1.98	11.3
Energy (kcal)	331	309	307	361- 363
Minerals (mg/100g)				
Phosphorus	290	188	280	296
Magnesium	81	147-228	82	137
Potassium	250	144	-	307
Sodium	4.6	4.6	-	10.9
Calcium	31	27	20-22	42
zinc	2.4	0.7	3.0	3.1
Iron	2.8	0.5-5.0	5.0-18.6	8.0
Manganese	0.6	1.1-3.3	0.96	1.15

copper	2.4	1.6	0.6	1.06

Source: (Ramashia, Mashau, and Onipe, 2021)

2.4.2 Recommended daily intake of nutrients in children under five

According to the WHO and FAO, children aged 6 to 59 months require sufficient daily intake of essential nutrients to support normal growth, cognitive development, and immune function. Key nutrients include protein, iron, zinc, calcium, and energy, which are critical for tissue repair, bone development, and the prevention of malnutrition. The amount of porridge consumed also influences nutrient adequacy, with older children generally consuming larger portions than younger ones. Flour from germinated millet (FGM) provides a valuable protein source, but it may meet the recommended dietary allowance (RDA) only for older infants, while younger infants may not receive adequate protein from FGM alone. Supplementing millet flour with spirulina produces millet–spirulina composite flour (MSCF), which enhances protein quality and helps balance nutrient intake across different age groups. MSCF significantly increases protein content, ensuring sufficient nutrition for children aged 6 to 59 months, as illustrated in the comparative protein contribution table of FGM and MSCF (Table 4).

Table 4: Recommended Nutrient Intakes and Protein Contribution for Children

Age (months)	**Recommended Porridge Quantity**	**Flour/Day (g)**	**Energy (kcal/day)**	**Protein RDI (g/day)**	**Protein from GMF (g)**	**% RDA Met (GMF)**	**Protein from MSCF (g)**	**% RDA Met (MSCF)**	**Iron RDI (mg/day)**	**Zinc RDI (mg/day)**	**Calcium RDI (mg/day)**
6	2 tsp × 2	20	1,000	13	1.99	21%	3.15	33%	7.0	3.0	260
6–8	½ cup × 2	100	1,200	15	9.97	95%	15.76	150%	8.0	4.0	500
9–12	½ cup × 3	150	1,500	19	14.96	119%	23.64	188%	10.0	5.0	700
13–24	1 cup × 2	200	1,200–1,300	19	19.94	105%	31.52	166%	10.0	5.0	700
25–59	1 cup × 3	300	1,400–1,500	19	29.91	157%	47.28	249%	11.0	5.0	700

Source: (FAO and WHO, 2014)

2.4.3 Thresholds for Declaring a Food “Rich in” or “Source of” Key Nutrients

According to the Codex Alimentarius Guidelines on Nutrition and Health Claims (CAC/GL 23-1997, revised 2013) and the Uganda National Bureau of Standards (UNBS, US EAS 803:2019), foods are classified as “a source of” or “rich in” a nutrient based on the proportion of the Recommended Dietary Allowance (RDA) they provide per 100 g or per serving. A food qualifies as “a source of protein” if protein contributes at least 12% of the total energy value, and as “rich in protein” if protein contributes 20% or more of total energy (Table 5). For minerals such as iron and calcium, a product is considered “a source of” the nutrient when it provides at least 15% of the RDA, and “rich in” or “high in” if it supplies 30% or more of the RDA per 100 g or per serving. These standards inform nutritional labeling and health claims internationally and have been adopted in Uganda to ensure consumers receive accurate information.

Table 5: Thresholds for Declaring a Food "Rich in" or "Source of" Key Nutrients

Nutrient	Nutrient Claim	Threshold (per 100 g of food)	Reference Source
Protein	"Source of protein"	≥12% of total energy from protein	Codex CAC/GL 23-1997
Protein	"Rich in protein" / "High in protein"	≥20% of total energy from protein	Codex CAC/GL 23-1997
Iron	"Source of iron"	≥15% of the RDA per 100 g or serving	Codex CAC/GL 23-1997; UNBS EAS 803:2019
Iron	"Rich in iron" / "High in iron"	≥30% of the RDA per 100 g or serving	Codex CAC/GL 23-1997; UNBS EAS 803:2019
Calcium	"Source of calcium"	≥15% of the RDA per 100 g or serving	Codex CAC/GL 23-1997; FAO/WHO, 2013
Calcium	"Rich in calcium" / "High in calcium"	≥30% of the RDA per 100 g or serving2	Codex CAC/GL 23-1997; FAO/WHO, 2013

Source: (EAS 803:2019; FAO/WHO, 2013; Codex CAC/GL 23-1997)

2.4.4. The local millet varieties in Uganda

Northern and Eastern Uganda host a wide variety of local millet cultivars, including *Engeyi, Seremi 1–3, P224, Gulu-E, U-15, Obeete, Emodingoit, Emiroit, Serere 14, Sec 695, Eding, Engom*, *Elaba*, *Okiring,* and several *Naromil* types *(Naromil 1–5*) (Kasule *et al*., 2023). Among the improved varieties released by the National Semi-Arid Resources Research Institute (NaSARRI), *Seremi 2* and *Pese 1* are the most widely cultivated, while local varieties such as *Eserait, Etiyo, Otunduru, Emiroit, Obeet,* and *Okwangapel* continue to be grown in these regions (Kasule *et al*., 2023). Although millet cultivation is less extensive in Western and Central Uganda, local varieties from Bunyoro (e.g., *Okiring*, *Eding*) and Ankole (e.g., *Emiroit, Engeyi*) enhance the country's genetic diversity and are well adapted to regional agro-ecological conditions, including rainfall distribution and soil types (Kasule *et al*., 2023). When choosing millet varieties, farmers prioritize traits such as high yield, pest and disease resistance, tolerance to shattering, early maturation, compact inflorescence, grain size, storage longevity, brewing quality, aroma, market appeal, and taste (Kasule *et al*., 2023; Adikini *et al*., 2021).

Desired traits in millet include high yield, resistance to lodging, adaptability to diverse agro-ecological conditions, and favorable malting and grain quality (Kasule *et al*., 2023). These characteristics explain why certain varieties remain popular in Northern and Eastern Uganda, as they are preferred for preparing traditional foods such as Ugandan *ugali* (*kalo*), thick porridge, and other local dishes (Kasule *et al*., 2023). For example, thick porridge made from the Gulu-E variety is known locally as "*Atapa*" in the Teso region and "*kwon kal*" among the Luo of eastern Gulu district (Kasule *et al*., 2023). Common cultivars include *Kal Atar* and *Okello Chiba* in Lira, *Ajuko Manyige* in Nwoya, and *Kaguma* in Bushenyi. Farmers in Uganda, as well as in parts of

East Africa like Kenya and Tanzania, have widely adopted improved varieties such as *Seremi 2 (U15) and Pese 1 (P224)* due to their favorable traits, including high tiller numbers, large grain size, attractive coloration, and early maturity (Kasule *et al*., 2023). These improved varieties are also adaptable across multiple regions, extending their cultivation beyond Northern and Eastern Uganda.

2.4.5. Anti-nutritional components in millet

Millet contains several anti-nutritional factors that limit nutrient availability, including phytates, tannins, oxalates, protease inhibitors, saponins, and lectins (Jan, Kumar, Yadav, Ahmed, Thakur, Chauhan and Dhaliwal, 2022; McNeil, 2013). Most of the total phosphorus in plant tissues occurs as phytic acid, also known as myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate (Sheethal *et al*., 2022). Phytic acid serves as the main storage form of phosphorus, particularly concentrated in the bran and germ of seeds, legumes, oilseeds, and nuts (Samtiya, Aluko and Dhewa, 2020; Lott, Ockenden, Raboy and Batten, 2000). Its negatively charged molecules bind to positively charged minerals such as zinc, iron, magnesium, and calcium, forming insoluble complexes that are poorly absorbed in the gastrointestinal tract. Approximately 50 to 80% of the phosphorus in seeds exists in this bound form, which limits mineral bioavailability (Samtiya, Aluko and Dhewa, 2020). This chelation process can lead to mineral deficiencies, especially zinc deficiency, and may impair bone development, immune function, and overall growth (Bohn, Meyer and Rasmussen, 2008).

Tannins, on the other hand, interact with protein carbonyl groups via their hydroxyl groups to form both reversible and irreversible tannin-protein complexes (Molino, Francino and Henares, 2023). These complexes interfere with protein digestion, decreasing the availability of essential amino acids, lowering growth rates, reducing iron absorption, and diminishing dietary efficiency (Samtiya, Soni, Chawla, Poonia, Sehgal and Dhewa, 2021). High tannin intake can irritate the gastrointestinal tract, though safe levels vary by food type, processing methods, and species, and are generally below 5% of total dietary intake in humans (UNBS, 2017). Binding of tannins to proteins also produces an astringent taste that reduces food palatability (Samtiya *et al*., 2021). In legumes, tannins are mainly concentrated in the seed coat and can inhibit digestive enzymes, further decreasing protein digestibility when consumed in large amounts (Samtiya *et al*., 2021).

Oxalates in foods can bind with minerals such as calcium, potassium, and sodium to form salt compounds, which may be either insoluble, like calcium oxalate, or soluble, such as potassium and sodium oxalates (Salgado, Silva, Figueira, Costa and Albuquerque, 2023). Calcium oxalate can further react with other compounds to produce forms such as calcium phosphate, calcium carbonate, calcium sulfate, calcium chloride, and calcium gluconate (Akbar, 2022). These crystalline structures, particularly calcium oxalate, contain substantial amounts of bound calcium, reducing its bioavailability and limiting absorption in the human body (Vavrusova and Skibsted, 2014). The extent of calcium absorption is influenced by the oxalate-to-calcium ratio: high oxalate with low calcium decreases bioavailability, whereas low oxalate with high calcium enhances absorption (Akbar, 2022; Palgi, 2005).

Protease inhibitors are naturally occurring plant compounds that reduce enzyme activity through protein–protein interactions, making them an important subject for research (Bonturi, Silva Teixeira, Rocha, Valente, Oliveira, Filho and Oliva, 2022). By binding to enzyme active sites, these inhibitors suppress catalytic function. Structural regions, including loop sequences and N- or C-termini, are crucial for enzyme inhibition, facilitating inhibitor–enzyme binding, as shown in Figure 1 (Samtiya *et al*., 2020). A bifunctional protease inhibitor in millet deactivates enzymes responsible for digesting proteins and starches by forming trimeric complexes with trypsin and α-amylase, respectively (Pandey, Singh, Srivastava, Zanwar, Dar, Singh and Lal, 2023). Cereals like millet contain lectins, protease inhibitors, and α-amylase inhibitors, which can reduce mineral availability, nutrient absorption, and protein digestibility (Adebo, Njobeh, Gbashi, Oyedeji, Ogundele, Oyeyinka and Adebo, 2022). However, processing techniques such as soaking, fermentation, and boiling can decrease these anti-nutritional compounds and improve nutrient bioavailability (Samtiya *et al*., 2020).

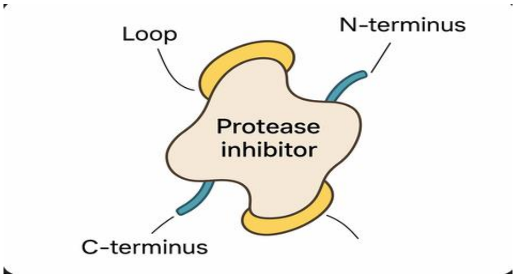

Figure 1: The loop regions and the N - or C termini (Samtiya *et al*., 2020)

2.4.6 Common millet processing methods

2.4.6.1 Soaking

Millet grains are soaked in either warm or cold water, typically between 30°C and 70°C, for a period ranging from 6 to 24 hours. After this process, the grains are rinsed, drained, and dried prior to milling (Nhara *et al*., 2024). Soaking promotes the breakdown and migration of certain compounds and minerals from the grain into the soaking medium (Kate and Singh, 2021). It plays a vital role in lowering anti-nutritional constituents such as phytic acid and tannins (Abioye *et al*., 2022; Shrestha, Six, Dahal, Marks and Meierhofer, 2020). Because phytic acid, tannins, and polyphenols are water-soluble, they tend to leach out during soaking, leading to a substantial decline in their concentration within the grains (Kate and Singh, 2021; Lestienne, Mouquet-Rivier, Icard-Verniere, Rochette and Treche, 2005). The soaking process also stimulates natural enzymes like phytase, which degrade phytic acid into lower forms of inositol phosphates, freeing minerals such as iron, zinc, and calcium and thereby enhancing their bioavailability (Sheethal *et al*., 2022). The degree of reduction in anti-nutritional compounds depends largely on soaking time, temperature, and pH. Higher temperatures and prolonged soaking increase both enzyme activity and leaching efficiency. However, small losses of water-soluble minerals, including sodium, phosphorus, and iron, may occur during the process. In general, soaking is an effective pre-treatment technique that minimizes anti-nutritional factors and promotes mineral availability in millet grains (Nhara *et al*., 2024).

2.4.6.2 Germination

Millet grains are first soaked for about 12 hours under controlled conditions and then allowed to germinate for approximately 48 hours. Extending germination beyond 72 hours may result in considerable nutrient loss from the grains (Abioye *et al*., 2022). A 48-hour germination period has been shown to markedly lower the levels of anti-nutritional factors, particularly phytic acid and tannins, while enhancing the mineral profile of the grains (Kate and Singh, 2021). Studies have indicated that during this process, protein content increases from around 9.3% to 10.6%, and total sugar concentration rises from 2.2% to 5.5% (Tumwine, Atukwase, Tumuhimbise, Tucungwirwe and Linnemann, 2019). Meanwhile, total phenolic compounds drop by from 4.17

mg/100 g, and tannins decline by from 28.33 mg/100 g (Abioye *et al*., 2022). The phytic acid content of millet, which normally falls between 588 and 1382 mg/100 g, can be reduced by more than half when germinated at ambient temperature (25 ± 4°C) for 24 hours (Samtiya *et al*., 2021).

The decline in these anti-nutritional factors during germination results from heightened enzymatic and metabolic activities in the seed. As the grains absorb water, they activate hydrolytic enzymes such as phytase, tannase, and polyphenol oxidase, which degrade complex anti-nutrients into simpler forms that are easier for the body to absorb (Sheethal *et al*., 2022). Phytase, in particular, breaks down phytic acid into lower inositol phosphates and free phosphate, freeing minerals like iron, zinc, and calcium, and improving their absorption efficiency (Abioye *et al*., 2022). Similarly, tannase and polyphenol oxidase act on tannins and phenolic compounds, reducing their tendency to form complexes with proteins and minerals. In addition, increased respiration during sprouting utilizes stored carbohydrates and stimulates protein synthesis, which further reduces anti-nutrient concentration through metabolic dilution (Sheethal *et al*., 2022). Collectively, these physiological and biochemical changes explain the marked reduction in phytic acid, tannins, and phenols following germination (Abioye *et al*., 2022; Sheethal *et al*., 2022; Samtiya *et al*., 2021).

2.4.6.3 Fermentation

Fermentation is a biochemical process that relies on the activity of microorganisms to improve food stability and enhance nutrient availability (Jan, Kumar, Yadav, Ahmed, Thakur, Chauhan and Dhaliwal, 2022; McNeil, 2013). In millet, spontaneous fermentation conducted without inoculating specific starter cultures typically occurs over 12 to 48 hours and is mainly facilitated by lactic acid bacteria (LAB). These microorganisms lower the pH of the substrate and increase its acidity, creating favorable conditions for nutrient enhancement and microbial safety (Abioye *et al*., 2022). The process elevates the levels of free amino acids and essential amino acids such as lysine, methionine, and tryptophan, thereby improving the overall protein quality of the fermented product (Nhara *et al*., 2024).

During fermentation, antinutritional phytic acid reduced by approximately 20%, depending on temperature and microbial activity (Abioye *et al*., 2022). This reduction is attributed to enzymatic degradation by LAB, which produce hydrolytic enzymes such as phytase, tannase, and protease. Phytase catalyzes the breakdown of phytic acid into lower inositol phosphates, liberating bound minerals like iron, zinc, and calcium. Tannase hydrolyzes tannins, lowering their ability to form complexes with proteins and minerals, while microbial proteases deactivate protease inhibitors, thus enhancing protein digestibility (Samtiya *et al*., 2020). The acidic environment generated during LAB fermentation promotes these enzymatic reactions and inhibits the growth of spoilage microorganisms, collectively leading to a 30% decrease in antinutrient levels after 12 to 48 hours of fermentation. In addition to improving nutrient bioavailability, fermentation also contributes to desirable sensory attributes such as improved taste and aroma, thereby enhancing the acceptability and overall nutritional quality of millet-based foods (Muyanja, Kikafunda, Narvhus, Helgetun and Langsrud, 2003).

2.4.6.4 Malting

Malting is a sequential process comprising soaking, germination, drying, milling, and sieving steps, designed to improve both the nutritional and functional quality of millet (Abioye *et al*., 2022). Through these stages, the digestibility of starch is enhanced, sensory characteristics such as taste and texture are improved, and concentrations of antinutritional factors like phytic acid and tannins are markedly reduced. Empirical studies indicate that malting can decrease phenolic content by about 23.9% after 72 hours and by approximately 45.3% after 96 hours (Nhara et al., 2024; Abioye *et al*., 2022). The process also boosts mineral bioavailability and increases the nutritional density of millet, making it a more valuable ingredient for traditional foods and beverages commonly consumed in rural and cultural settings (Abioye *et al*., 2022; Amadou, Gbadamosi and Le, 2011)

The decline in antinutritional compounds during malting is primarily attributed to the activation of endogenous enzymes and physiological changes occurring throughout soaking and germination (Abioye *et al*., 2022). Soaking initiates the activity of enzymes such as phytase and tannase, which remain active during germination (Nhara *et al*., 2024). Phytase catalyzes the hydrolysis of phytic acid into lower inositol phosphates and inorganic phosphate, thereby liberating bound minerals including iron, zinc, and calcium, and improving their absorption efficiency (Samtiya *et al*., 2020). Likewise, tannase and polyphenol oxidase act on tannins and phenolic compounds, minimizing their capacity to bind with proteins and minerals (Abioye *et al*., 2022). Additionally, germination triggers metabolic respiration and protein synthesis within the grains, which not only dilutes the concentration of antinutritional factors but also enhances the availability of amino acids (Nhara *et al*., 2024). Collectively, these enzymatic and metabolic activities account for the significant reduction in phenolic compounds and other antinutrients observed during the malting process (Abioye *et al*., 2022).

2.4.6.5 Roasting

Millet grains are subjected to dry heat treatment at temperatures between 120°C and 180°C for about 3 to 15 minutes (Nhara *et al*., 2024). Roasting improves the flavor, aroma, and overall palatability of millet products, enhancing consumption and nutritional efficiency when used in human or animal diets (Tumwine *et al*., 2019). This process also decreases levels of antinutritional compounds such as phytates and tannins, while markedly improving protein digestibility from approximately 22.3% to 60.1%. For instance, heating millet at 120 °C has been reported to lower phytate content by nearly 34.9% (Nhara *et al*., 2024). Despite these benefits, roasting can lead to minor losses of heat-sensitive nutrients such as certain proteins and sugars, largely due to the Maillard reaction and other thermal alterations (Tumwine *et al*., 2019).

The decline in antinutritional factors during roasting results mainly from thermal degradation and heat-induced molecular transformations (Abioye *et al*., 2022). Elevated temperatures denature heat-labile substances like phytates and tannins, altering their structural integrity and reducing their capacity to chelate minerals or bind with proteins (Nhara *et al*., 2024). Additionally, the heat softens and partially disrupts cell wall structures, which facilitates the release of minerals and enhances their bioavailability (Abioye *et al*., 2022). Denaturation of proteins further minimizes interactions with antinutritional agents, such as tannins and protease inhibitors, thereby promoting better protein digestibility (Nhara *et al.*, 2024). Collectively, these mechanisms make roasting an efficient technique for reducing antinutrient levels and enhancing nutrient accessibility in millet-based food products (Abioye *et al.*, 2022).

2.5 Utilization and composition of Spirulina

2.5.1 Overview of Spirulina

Spirulina (*Arthrospira platensis*) is a filamentous cyanobacterium (blue-green microalga) belonging to the phylum *Cyanobacteria*. It is globally valued for its exceptionally high protein content, balanced essential amino acid profile, and richness in vitamins, minerals, pigments, and bioactive molecules, which make it an important ingredient in functional and nutraceutical foods (Koli, Rudra Bhowmik and Pabbi, 2022). Its spiral, filamentous morphology enables buoyancy, allowing maximum exposure to sunlight for photosynthesis. Additionally, its metabolic system efficiently utilizes inorganic nutrients such as carbon, nitrogen, and minerals to sustain rapid growth (AlFadhly *et al.*, 2022). Spirulina naturally thrives in alkaline, warm aquatic environments, typically at temperatures of 35 to 37 °C and pH values ranging from 9.5 to 10.5 (AlFadhly *et al.*, 2022; Aljobair, Albaridi, Alkuraieef and AlKehayez, 2021). Such conditions limit the growth of competing microorganisms, giving *Arthrospira* a selective ecological advantage. The species is abundant in tropical and subtropical regions, especially in saline lakes such as Lake Chad in Africa and Lake Texcoco in Mexico (AlFadhly *et al.*, 2022). In Uganda, natural conditions are generally less favorable for large-scale *Spirulina* cultivation. Nonetheless, local initiatives like the Spirulina Development Institute (SDI) have introduced controlled cultivation systems and farmer training programs. These efforts aim to improve local nutrition, enhance food security, and create sustainable livelihood opportunities by promoting cost-effective production and community-based microalgae farming (Blue Atlas Project).

2.5.2 Utilization of Spirulina

Spirulina has been utilized as a fortifying ingredient in a variety of food products to improve their nutritional value, functional properties, and sensory characteristics (Ziena, Rozan and Ghozlan, 2020). It has been successfully incorporated into foods such as wheat-based bread, corn–soy blends (CSB), and green pasta, often at substitution levels between 0% and 15% (Aljobair *et al.*, 2021) (Table 6). Research has demonstrated favorable nutritional and sensory outcomes from the addition of Spirulina to corn–soy blends and pasta formulations (Koli *et al.*, 2022; Othoo *et al.*, 2021). However, limited studies have explored the integration of Spirulina into millet-based products, particularly those using flour from germinated millet, to assess changes in nutritional composition and functional quality.

A substitution rate of around 10% is generally reported to achieve good consumer acceptance while maintaining desirable flavor and texture attributes. The unique green-blue color of Spirulina-enriched foods arises from its natural pigments chlorophyll (green), phycocyanin (blue), and carotenoids such as β-carotene and zeaxanthin (yellow–orange). Increasing Spirulina concentration enhances color intensity and raises protein content because of its exceptionally high protein level (60 to 70%) and balanced essential amino acid composition (Koli *et al.*, 2022). Nevertheless, excessive inclusion may adversely affect sensory appeal if not carefully optimized.

Table 6: Proportion of Spirulina used for enhancement of nutritional profile of foods

Foodstuff	Major ingredients	Proportion of spirulina used (w/w)
Green pasta	Durum wheat semolina, carboxymethyl cellulose	2% to 15%
Corn soy blend	Whole maize, whole soy bean	0.40%
Bread	Wheat flour	2% to 8%

Sources: (Saharan and Jood, 2017 ; Othoo *et al*., 2021; Koli *et al*., 2022)

2.5.3 Nutritional Composition of Spirulina

A range of microalgae species, such as *Spirulina*, *Chlorella vulgaris,* and *Odontella aurita*, have been designated as "Generally Recognized as Safe" (GRAS) for human consumption by both the European Commission and the U.S. Food and Drug Administration. This classification is based on their historical use in foods and extensive evidence supporting their safety (Fernandes, Campos, Serra, Fidalgo, Almeida, Casas, Toubarro and Barros, 2023; Montevecchi, Santunione, Licciardello, Köker, Masino and Antonelli, 2022). Among the various *Spirulina* species, *Arthrospira platensis*, *A. maxima*, and *A. fusiformis* are the most widely utilized in food applications. The present study employed *A. platensis* because of its well-established nutrient composition, ease of cultivation, and verified safety profile (Fernandes *et al*., 2022). *Spirulina platensis* is a rich source of high-quality proteins, vitamins (such as B_{12} and provitamin A), polyunsaturated fatty acids, pigments (including phycocyanins and carotenoids), essential minerals, and phenolic compounds (Khandual, Sanchez, Andrews and De la Rosa, 2021) (Table 7). Its bioactive molecules such as zeaxanthin, astaxanthin, β-carotene, polyphenols, and chlorophyll exhibit antioxidant, anti-inflammatory, and immune-enhancing activities (Janda-Milczarek, Szymczykowska, Jakubczyk, Kupnicka, Skonieczna-Żydecka, Pilarczyk, Tomza-Marciniak, Ligenza, Stachowska and Dalewski, 2023). Moreover, the iron in Spirulina is highly bioavailable, supporting efficient mineral absorption even in the presence of dietary antinutritional factors (AlFadhly *et al*., 2022; Othoo *et al*., 2021; Peng, 2004).

Table 7: Nutritional composition of spirulina

Nutrients	Composition (g/100 g dry basis)
Carbohydrates	12.8-68.9
Proteins	60-70
Ash	1.8-6.1
Fibers	10.3
Iron	26.59
Calcium	107.83
Zinc	1.87

Niccolai *et al*. (2019), Othoo *et al*. (2021) & Koli *et al*. (2022)

CHAPTER THREE: MATERIALS AND METHODS

3.1 Experimental Design

A completely randomized factorial design (CRD) was used to examine how germination and varying levels of Spirulina substitution influence the nutritional quality, mineral profile, and sensory characteristics of millet-based composite flour intended for porridge preparation. The experimental design included two independent variables: germination status and percentage of Spirulina addition. Germination had two levels (non-germinated and germinated millet) while Spirulina was incorporated at six substitution levels of 0%, 2%, 4%, 6%, 8%, and 10%. Combining these factors produced eighteen treatment sets (3 × 6), each replicated three times (n = 3) to ensure experimental precision and reproducibility. Eight millet varieties (*Ekwangapel*, *Ekaama*, *Emoromoru*, *Ayuku Manyige*, *Odyera*, *Min Bel*, *Luk Caa*, and *Adyang Adyang*) were processed into flours from both germinated and non-germinated millet using the procedures outlined below. Germination was performed under controlled laboratory conditions to enhance nutrient bioavailability, while ungerminated millet served as the experimental control.

Spirulina powder, sourced from a certified supplier at Masinde Muliro University of Science and Technology in Nairobi, Kenya, millet flour was incorporated into spirulina powder at the six substitution levels stated above. The formulation followed the NutriSurvey (2007) optimization model to meet nutrient requirements for children aged 6 to 59 months. The composite flours were thoroughly mixed to ensure uniformity, sealed in airtight polyethylene bags, and stored at ambient temperature (25 ± 4°C) before further analysis (Sharma and Anurag, 2023). All formulations were analyzed for proximate composition, mineral concentration, and antinutritional factors using standardized AOAC (2019) analytical methods. The concentrations of key minerals like calcium, iron, zinc, and magnesium were determined using Atomic Absorption Spectrophotometry (AAS), while levels of phytates, oxalates, and tannins were measured through spectrophotometric methods. All analyses were conducted in triplicate to ensure precision and reliability of the results. The percentage contribution of protein to the Recommended Dietary Allowance (RDA) was computed based on the measured protein content of each formulation in comparison with WHO (2014) reference standards for children aged 6 to 59 months, enabling assessment of each flour's adequacy in meeting daily protein needs.

Sensory evaluation was performed by a panel of 90 untrained participants using a structured 9-point hedonic scale to rate parameters such as appearance, color, taste, flavor, texture, mouthfeel, and overall acceptability. Data from all determinations were analyzed statistically using Analysis of Variance (ANOVA) within a factorial CRD framework at a 95% confidence level ($p < 0.05$) to identify significant differences among treatments. Mean comparisons were made using Tukey's Honest Significant Difference (HSD) test, and all results were reported as mean ± standard deviation (SD).

3.2 Materials

A total of five kilograms of each of eight local millet varieties (*Ekwangapel, Ekaama, Emoromoru, Ayuku Manyige, Odyera, Min Bel*, *Luk Caa*, and *Adyang Adyang*) were procured from farmers in Northern and Eastern Uganda, specifically from Gulu, Lira, and Soroti Districts. Soroti supplied *Ekwangapel, Ekaama*, and *Emoro moru*; Gulu provided *Ayuku Manyige, Odyera, Min Bel*, and *Luk Caa*; and *Adyang Adyang* was obtained from Lira.

These millet varieties were selected based on their availability, local popularity, and frequent use as staple foods in the respective regions. The districts were purposively chosen due to their high millet production, strong cultural reliance on millet-based diets, and ongoing child malnutrition issues as reported in the Uganda Demographic and Health Survey (UDHS, 2022). Spirulina (*Arthrospira platensis*) powder, supplied in airtight packaging to prevent moisture uptake and oxidation, was sourced from Masinde Muliro University of Science and Technology in Nairobi, Kenya. Spirulina was selected as a fortificant owing to its high protein content (60 to 70%), balanced amino acid composition, and richness in essential micronutrients including iron, zinc, calcium, magnesium, and provitamin A. Its excellent digestibility, low anti-nutritional factor content, and proven efficacy in combating protein-energy malnutrition make it an ideal supplement to enhance the nutritional quality of millet-based complementary foods. All raw materials were transported to the Food Science Laboratory at Kyambogo University and stored at ambient temperature (25 ± 4°C) until further processing and analysis.

3.3 Sampling

A stratified random sampling approach was used to obtain representative millet samples. Each district served as a stratum, from which three parishes were randomly selected. Within each parish, three villages (Lamin Latoo in Nwoya, Gulu, Aleptong in Lira and Ojakai in Soroti) were chosen. For each millet variety, grains were procured from three different farmers per village and combined to form a single composite sample for that variety. This method ensured that each millet variety was represented by a homogeneous composite while capturing variability across farmers. The eight millet varieties were selected based on farmer preference, grain availability, and local use in porridge preparation, enabling comparisons between high-yielding improved varieties and traditional landraces.

3.4 The germination process

Fungi are generally unnecessary for millet germination and can be detrimental, as pathogenic species such as *Fusarium* and *Alternaria* may cause seed rot or seedling blight. Storage fungi, including *Aspergillus* and *Penicillium*, can also reduce seed viability under conditions of high moisture and temperature. Fungal growth competes with seeds for oxygen, inhibiting embryo development and reducing germination success. To ensure consistent sprouting, only mature, physiologically viable, and uniformly sized millet seeds were selected, while immature, damaged, or dormant seeds were excluded. To minimize fungal contamination, 500 g of millet grains per variety were germinated under controlled laboratory conditions. The grains were soaked overnight in 1,500 mL of water at ambient temperature (25 ± 4°C) (Sharma and Anurag, 2023 ; Ocheme and Chinma, 2008), drained, and evenly spread on a clean surface before being covered with a moist, sterile cloth. The grains were watered every 24 hours for 48 hours, providing adequate moisture, moderate temperature, oxygen, and hygienic conditions to promote germination while preventing fungal growth. The sprouted grains were then dried in a hot-air oven at 59°C for 8.5 hours (Figure 2).

3.4.1 Preparation of flours from non-germinated and germinated millet

Non-germinated and germinated millet grains from each variety were milled into flour using a high-speed grinder (Model I and 200, 2018; Mazzer, Italy) operating at 1,400–1,600 rpm for 3 minutes. The resulting flour was passed through a MICS 425 mesh (59.76 µm) to achieve uniform particle size and ensure homogeneity (Figures 2(a) and 1(b)). The flours were then packed in airtight polyethylene bags and stored at ambient temperature (25 ± 4°C) until further analysis.

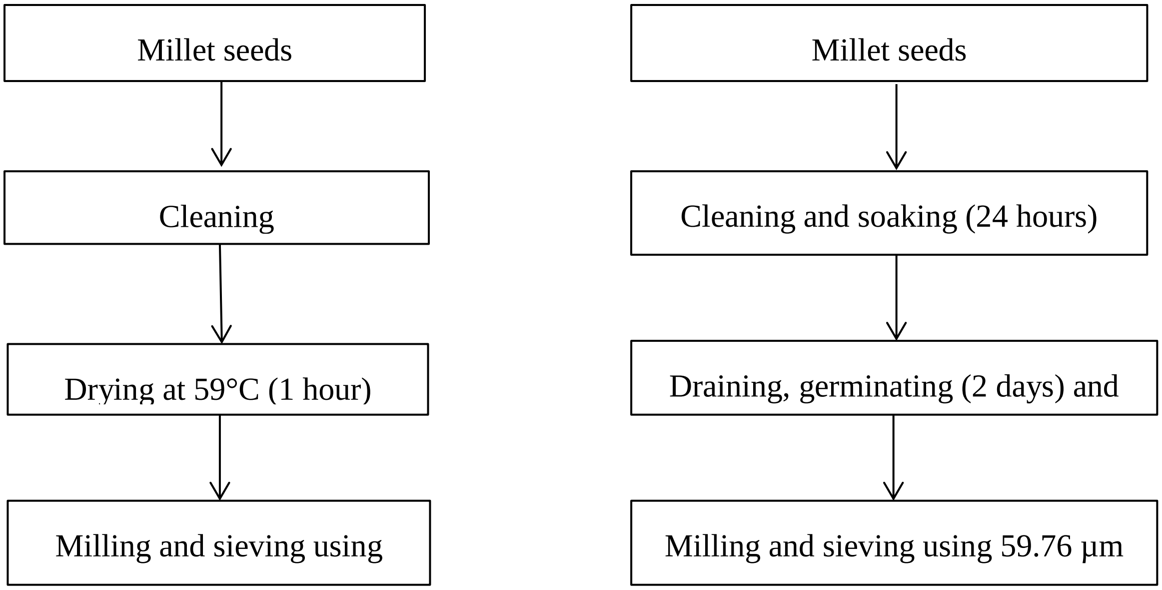

Figure 2: Production of flour from; (a) Non-germinated millet; (b) germinated millet

3.5 Determination of proximate composition

Analyses of proximate composition were performed at the Uganda Industrial Research Institute (UIRI) analytical laboratory using Analytical-grade chemicals. Flours from both non-germinated and germinated millet were analyzed using the same methods.

3.5.1 Determination of moisture

The moisture content of the millet flours was determined using the AOAC (2019) method, number 925.09. To determine the weight of the wet flour (W2), ten grams of the flour were weighed into a pre-weighed petri dish (W1) and then weighed again. A petri dish containing millet flour was dried at 105°C in an oven (Model: ED 115, GmbH, Germany) until its weight remained consistent. To calculate the weight of the dry millet flour (W3), the dried millet flour was chilled in a desiccator for 0.5 hour before being weighed again. Moisture content was calculated as follows;

$$\text{Moisture (\%)} = \frac{(W2)-(W3)}{(W1)} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

3.5.2 Determination of crude protein

The protein content of the millet flours was determined using AOAC (2019) method, number 992.15. One gram of flour was weighed into a digestion flask, after which 12 mL of sulfuric acid and one Kjeldahl catalyst tablet containing copper sulfate were added. A colorless solution was produced by digesting the homogenous mixture at 410°C. After cooling, the digest was poured into a 250 mL conical flask and diluted with 50 mL of distilled water. Additionally, 25 mL of boric acid, five drops of methyl red indicator, and 50 mL of 40% excess aqueous sodium hydroxide were added. After heating the mixture in the flask, the ammonia that was released was collected in a collection flask with 2% boric acid. Titrating the distillate with standardized 0.05N hydrochloric acid allowed for the determination of total nitrogen (%). By multiplying the nitrogen content by a conversion factor of 6.25, which establishes a relationship between nitrogen and protein, the protein content was calculated, as follows;

$$\text{Crude protein (\%)} = \frac{(V2-V1)\times M\times 14\times F}{W} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

V1 is the amount of HCl needed for the blank, and V2 is the amount needed for the sample. M = H2Cl's morality; 14 = nitrogen's atomic mass; W is the test sample's weight; The nitrogen conversion factor is F = 6.25.

3.5.3 Determination of crude fat

The crude fat content was determined using AOAC (2019) method, number 948.15. A pre-dried extraction thimble lined with Whatman No. 1 filter paper was filled with 5 g of flour. Sixty milliliters of petroleum ether were measured into a previously weighed dry aluminum cup, after which the thimble was covered and secured in the Soxhlet extraction apparatus. The thimble containing the flour was then submerged in hot water for 15 minutes. Following 30 minutes of extraction, compressed air was used to remove any remaining solvent. The extracted fat was dried at 105°C in an air oven for 30 minutes and subsequently weighed. Fat content was calculated using the formula below;

$$\text{Fat content (\%)} = \frac{(W3)-(W2)}{(W1)} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3$$

W1 is the initial sample weight; W2 is the extraction cup weight; and W3 is the extraction cup weight plus residue weight.

3.5.4 Determination of crude ash

The ash content was determined using AOAC (2019) method number 923.03. Three grams of flour were dried in an oven until a constant weight was achieved. The dried flour was then transferred to a muffle furnace (Carbolite-Gero 2132, UK) and heated at 550°C for four hours until it was converted to white ash. Ash content was calculated as follows;

$$\text{Ash (\%)} = \frac{(G2)-(G1)}{(W)} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..4$$

W is the initial sample weight; G1 is the crucible's tare weight; and G2 is the weight after ashing (sample plus crucible).

3.5.5 Determination of crude fibre

The crude fiber content was determined using AOAC (2019) method number 962.09. Two grams of flour were combined with one gram of Celite in a crucible. The mixture was defatted using 25 mL of acetone and then placed in a Fibre Hot Extraction Unit, where it was heated under reflux for 30 minutes in 200 mL of 1.25% sulfuric acid solution. The heated mixture was filtered using suction, and the residue was washed with hot water to remove any remaining acid. The residue was then refluxed and boiled in alkali for 30 minutes, filtered under vacuum, and washed thoroughly to remove any remaining alkali. The insoluble residue was dried at 130°C for two hours to a constant weight, cooled in a desiccator, and weighed. The amount of fiber was calculated as follows;

$$\text{Fibre content (\%)} = \frac{(W1)-(W2)}{(W0)} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.5$$

W0 is the sample weight; W1 is the weight of the crucible and residue; and W2 is the crucible weight.

3.5.6 Determination of carbohydrate

Carbohydrate content was determined by the difference method. That is the sum of all other proximate content substracted from 100.

3.6 Determination of mineral content

The amounts of calcium (Ca), iron (Fe), zinc (Zn), and magnesium (Mg) in flours from non-germinated and germinated millet were measured using an Atomic Absorption Spectrophotometer (AAS) (AAS System, Analyst 400, 2009, Perkin-Elmer, Singapore) in compliance with AOAC standard procedures (2000), method number 927.02. The minerals Ca, Fe, Zn, and Mg were produced in standard solutions. A calibration curve was constructed using the absorbance readings of each solution, as presented in the appendices. Three grams of flour were ashed for six hours at 550^{o}C in a Carbolite Gero 2132 muffle furnace (United Kingdom). Five milliliters of 20% hydrochloric acid were used to dissolve the remaining ash. The flours were inhaled into AAS, and the calibration curve for each mineral's concentration was utilized to calculate absorbance. The wavelengths (λ) of the minerals (Ca, Fe, Zn, and Mg) were measured at 442.7, 248.3, 213.9, and 285.2 nm, respectively.

3.7 Analysis of anti-nutritional factors

Anti-nutritional factors (phytates, oxalates and tannins) were analysed at the Natural Chemotherapeutics Research Institute (NCRI) laboratory.

3.7.1 Determination of phytate concentration

Wheeler and Ferrel's (1970) method was used to determine phytate. Two grams of flour from germinated and non-germinated millet were placed in a 125 mL Erlenmeyer flask, and 50 mL of 3% Trichloroacetic Acid (TCA) was added. The mixture was centrifuged, after which approximately 10 mL of the supernatant and 4 mL of ferric chloride were transferred into a centrifuge tube. The tube was heated in a water bath for 45 minutes, resulting in a clear supernatant. Following a second centrifugation, the clear supernatant was decanted. The residues were washed with 3% TCA, combined with sodium hydroxide, cooled, and their volume adjusted with distilled water. A 5 mL aliquot of the resulting solution was transferred into a 100 mL volumetric flask, and the volume was adjusted to the mark by adding 70 mL of water and 20 mL of potassium thiocyanate. Ferric nitrate standards were prepared at concentrations of 0, 0.1, 0.2, 0.3, 0.4, and 0.5 mg/L. Both the standards and flour samples were analyzed at a wavelength of 480 nm using an Analytik Jena Specord Plus UV-VIS Spectrophotometer. Phytate phosphorus was calculated from the measured iron values using the assumed 4:6 iron-to-phosphorus molecular ratio, based on the calibration curve provided in the appendices.

3.7.2 Determination of oxalate concentration

The oxalate concentration of the flours was determined using spectrophotometry (Naik, Patil, Aparadh and Karadge, 2014). A 0.5 g sample of flour was transferred into 30 mL of 0.25 N HCl and boiled in a water bath for 15 minutes. Subsequently, 50 mL of 0.25 N HCl was added. To 1 mL of the flour extract, 5 mL of 2 N H_2SO_4 and 2 mL of 0.003 M $KMnO_4$ were added, and the mixture was incubated at 37°C for 10 minutes. The oxalate concentration was calculated by multiplying the normality of $KMnO_4$ by the corresponding mass of the oxalate ion.

3.7.3 Determination of tannin concentration

The tannin was determined using the Folin and Ciocalteu technique. One gram of flour was boiled in water for 30 minutes and then filtered. Subsequently, 20 µL of the flour extract was mixed with 7.5 mL of distilled water, 0.5 mL of Folin reagent, and 1 mL of 35% sodium carbonate solution to make a total volume of 10 mL. The mixture was shaken and allowed to stand at room temperature for 30 minutes. The absorbance was measured at 725 nm, using water as the blank. A series of gallic acid standard solutions was prepared following the same procedure, and their absorbance values were compared to the blank. The tannin content was expressed as mg of gallic acid equivalent per gram of extract (mg GAE/g).

3.8 Formulation of the spirulina millet-based composite flour proportions

NutriSurvey software (2007) was used to generate and select the optimal levels of spirulina powder to incorporate into flour from germinated millet. Based on the software recommendations, spirulina was incorporated at substitution rates ranging from 0 to 10%, which were adopted as the optimal inclusion levels (Table 8).

Table 8: Proportions (%) of germinated millet flour and spirulina powder for formulation

Supplementation levels (%)	
Millet flour (MF)	**Spirulina powder (SP)**
100	0
98	2
96	4
94	6
92	8
90	10

3.9 Development of composite flours

Three superior local millet varieties (*Odyera*, *Ekaama*, and *Ekwangapel*) were selected for the development of composite flours based on their relatively high protein content, as determined from the proximate analysis conducted in this study (*Odyera*: 9.97%, *Ekaama*: 9.59%, *Ekwangapel:* 9.44%). These varieties were considered superior in terms of protein contribution because protein levels above 9% are regarded as high for millet flours and suitable for enhancing the nutritional quality of complementary foods for children (AOAC, 2019; FAO, 2013). Flour from germinated millet of the selected varieties were supplemented with spirulina powder at varying levels (0, 2, 4, 6, 8, and 10%) as shown in Table 8.

3.10 Determination of protein in composite flour

The protein content of the spirulina-millet based composite flours was confirmed using AOAC (2019) method, number 992.15. One gram of composite flour was weighed into a digestion flask, after which 12 mL of sulfuric acid and one Kjeldahl catalyst tablet containing copper sulfate were added. A colorless solution was produced by digesting the homogenous mixture at 410^{o}C. After cooling, the digest was poured into a 250 mL conical flask and diluted with 50 mL of distilled water. Additionally, 25 mL of boric acid, five drops of methyl red indicator, and 50 mL of 40% excess aqueous sodium hydroxide were added. After heating the mixture in the flask, the ammonia that was released was collected in a collection flask with 2% boric acid. Titrating the distillate with standardized 0.05N hydrochloric acid allowed for the determination of total nitrogen (%). By multiplying the nitrogen content by a conversion factor of 6.25, which establishes a relationship between nitrogen and protein, the protein content was calculated, as follows;

$$\text{Protein content (\%)} = \frac{(V2-V1)\times M\times 14\times F}{W} \times 100\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

V_{1} = Volume of HCl required for the blank; V_{2} = volume of HCl required for the sample; M = morality of HCl; 14 = atomic mass of nitrogen; W = weight of test sample; F = 6.25 = Nitrogen conversion factor

3.11.Determination of iron in composite flour

The amount of iron (Fe) in spirulina millet-based composite flours was measured using an Atomic Absorption Spectrophotometer (AAS) (AAS System, Analyst 400, 2009, Perkin-Elmer, Singapore) in compliance with AOAC standard procedures (2000), method number 927.02. The iron was produced in standard solutions. A calibration curve was created using the absorbance readings for every solution as below. Three grams of flour were ashed for six hours at 550^{o}C in a Carbolite Gero 2132 muffle furnace (United Kingdom). Five milliliters of 20% hydrochloric acid were used to dissolve the remaining ash. The flours were inhaled into AAS, and the calibration curve for each mineral's concentration was utilized to calculate absorbance. The wavelength (λ) of the iron was measured at 248.3 nm.

3.12 Determination of beta-carotene

The β-carotene content in spirulina millet-based composite flour was quantified using spectrophotometry. Ten grams of macerated flour were placed in a conical flask, and fifty milliliters of 95% ethanol were added. The flask was incubated in a water bath maintained at 80°C for 20 minutes to facilitate extraction. After cooling, the supernatant was decanted, and the original volume of the extract was noted. Subsequently, twenty-five milliliters of petroleum ether were added along with fifteen milliliters of distilled water, and the top petroleum ether layer, containing the β-carotene, was transferred to a 250 mL conical flask, while the aqueous layer was drained. The extraction of the aqueous layer was repeated with ten milliliters of petroleum ether until the extract attained a distinct yellow color. The petroleum ether extract was further washed with fifty milliliters of 80% ethanol to remove any remaining impurities. The final extract was analyzed using a spectrophotometer at 436 nm. The concentration of β-carotene was calculated using the Beer-Lambert law, which relates the absorbance of the solution to the concentration of the absorbing compound and the path length of the cuvette, thereby providing the β-carotene content in the sample.

3.13 Production of porridge from spirulina millet-based composite flour

Spirulina millet-based composite flours were reconstituted into porridge (Figure 3). Sixty grams of the flour was added to 500 mL of boiled water. The mixture was agitated on the hotplate for 10 to 15 minutes to obtain thick porridge and 24 g of sugar was added to generate sweet flavour. Same quantity of sugar and water was maintained in all porridge preparations using different formulations.

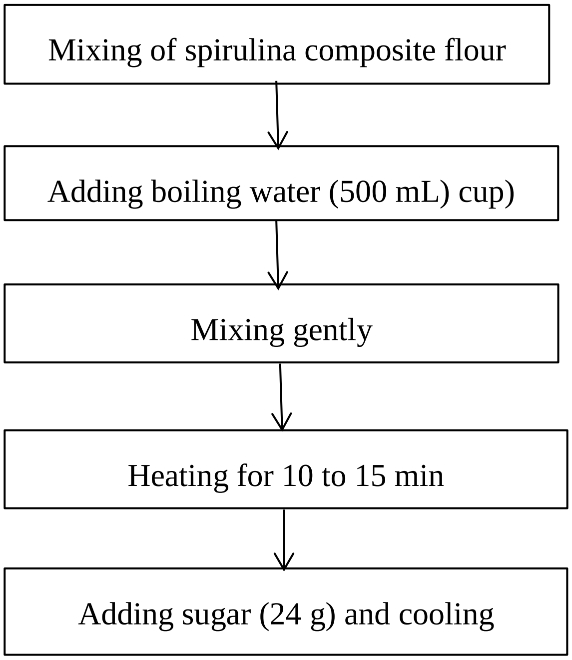

Figure 3: Production of porridge from spirulina millet-based composite flour

3.14 Sensory evaluation

Porridge was made using various ratios of spirulina and millet and evaluated for sensory qualities. A total of 90 mothers, who were not trained as panelists, participated in the assessment. The evaluation took place in Acholi Quarter, Banda B1, Kampala, within the local community. Sensory attributes (appearance, color, flavor, mouthfeel, taste, texture, and overall acceptability) were rated on a 9-point hedonic scale (Meilgaard, Carr and Civille, 1999; WHO and UNICEF, 2008). Panelists were provided with bottled water to cleanse their palates between tastings (Wacoo *et al*., 2019).

3.15 Data analysis

The protein, iron, and β-carotene levels of the three top-performing composite flours, along with the proximate composition, mineral content, and anti-nutritional factors of eight local millet varieties, were determined in triplicate (n = 3). Results are presented as mean ± standard deviation. Data analysis was performed using IBM SPSS version 23. Two-way ANOVA followed by Tukey's post hoc test was applied for multiple comparisons, with significance set at $p < 0.05$. Relationships among beta-carotene, protein, iron, and sensory attributes of porridge prepared from spirulina-millet composite flour were explored through Principal Component Analysis (PCA). Preference mapping was used to group samples and identify the most preferred formulation.

3.16 Ethical considerations

Ethical clearance for the study and consent from participating mothers was obtained from Clarke International University Research Ethics Committee (CIU-REC), reference number CLARKE-

2023-836. Additionally, a research permit was granted by the Uganda National Council for Science and Technology (UNCST), reference A432ES.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Proximate composition of non-germinated and germinated millet flours

4.1.1 Moisture

Flour from non-germinated millet had the highest moisture content in the *Min bel* variety (11.82%) and the lowest in *Emoro moru* (7.55%). Similarly, flour from germinated millet had the highest moisture content in the *Min bel* variety (10.08%) and the lowest in *Emoro moru* (6.98%) (Table 9). Generally, flour from non-germinated millet had reduced moisture content (10.00%) compared to germinated millet (8.69%), indicating that germination significantly decreased ($p < 0.05$) moisture content, with a reduction of 13.1%. Low moisture content is a desirable attribute for flours intended for complementary foods for children aged 6 to59 months, as it enhances product quality, stability, reduces the risk of microbial contamination and spoilage, and ensures safer product (Ojo, Akintayo, Faleye, Shittu and Adeola, 2017).

The reduction in moisture content following germination could be attributed to the drying process used to stabilize the germinated grains and prolong shelf life (Kumar, Kaur, Gupta, Gat and Kumar, 2021). Enzymatic and metabolic activities during germination also contribute to moisture reduction as amylase enzymes hydrolyze complex starches (polysaccharides) into simpler sugars (monosaccharides), releasing bound water and leading to drier grains (Sruthi and Rao, 2021). The moisture content values obtained in this study were within the recommended limits of the Uganda Standard (max. moisture content, 13.5%) (US EAS 89:2017), indicating that the flours are safe for storage. The mean moisture content values (8.95 to 10.00%) obtained in this study are in agreement to those reported by Ramashia *et al*. (2018), who found moisture contents ranging from 7.88 to 9.38% in millet grains and 9.17 to 11.67% in millet flours. The differences in moisture content among millet varieties can be attributed to their genetic composition, grain structure, and environmental growing conditions, including soil type and humidity (Qaisrani, Murtaza, Khan, Bibi, Iqbal, Azam, Hussain and Pasha, 2019)

Millet varieties	**Moisture content**		**Protein content**		**Fat content**		**Ash content**		**Fiber content**		**Carbohydrates**	
	Non-germinated	**Germinated**	**Non-germinated**	**Germinated**	**Non-germinated**	**Germinated**	**Non-germinated**	**Germinated**	**Non-germinated**	**Germinated**	**Non-germinated**	**Germinated**
Ekwangapel	10.36±0.04^{bcA}	8.19±0.01^{dB}	7.66±0.05^{bcB}	9.44±0.04^{bA}	1.25±0.01^{bB}	2.25±0.01^{dA}	2.31±0.03^{cdA}	2.19±0.01^{cB}	2.01±0.07^{bcA}	1.68±0.03^{cB}	76.41±0.02^{cdA}	75.24±0.06^{bB}

Table 9: Proximate composition (%) in flour from non-germinated and germinated millet varieties

Ekaama	8.19±0.74^{dA}	7.78±0.02^{eB}	7.97±0.05^{bB}	9.59±0.06^{abA}	1.14±0.01^{cdB}	2.46±0.01^{bcA}	**3.67±0.01^{aA}**	**2.87±0.01^{aB}**	**2.35±0.01^{aA}**	**1.88±0.01^{aB}**	76.35±0.53^{dA}	75.40±0.07^{dB}
Emoro moru	**7.55±0.03^{dA}**	**6.98±0.14^{fB}**	**6.89±0.05^{fB}**	8.78±0.06^{eA}	1.21±0.01^{cB}	**2.03±0.21^{fA}**	2.10±0.01^{eA}	**1.68±0.13^{fB}**	2.14±0.01^{abA}	1.59±0.04^{dB}	**80.12±1.79^{aA}**	**79.02±0.11^{aB}**
Ayuku manyige	9.34±0.04^{cA}	8.20±0.11^{dB}	7.29±0.02^{dB}	8.96±0.05^{dA}	1.05±0.04^{eB}	**2.61±0.01^{aA}**	3.00±0.01^{abA}	2.18±0.02^{cB}	2.13±0.06^{abA}	1.63±0.03^{cdB}	77.19±1.14^{bA}	76.17±0.07^{cB}
Odyera	10.62±0.06^{bA}	9.72±0.14^{bB}	**8.30±0.02^{aB}**	**9.97±0.07^{aA}**	**1.27±0.01^{aB}**	2.58±0.04^{abA}	2.45±0.02^{cA}	2.35±0.01^{bB}	1.91±0.03^{cA}	1.54±0.01^{eB}	**75.43±2.44^{fA}**	**73.85±0.16^{eB}**
Min bel	**11.82±0.01^{aA}**	**10.08±0.02^{aB}**	7.48±0.01^{cB}	9.29±0.04^{bcA}	1.06±0.01^{deB}	2.19±0.01^{eA}	2.67±0.75^{bA}	2.08±0.02^{dB}	**1.64±0.01^{eA}**	**1.28±0.01^{fB}**	76.33±0.76^{eA}	75.09±0.06^{deB}
Luk caa	11.43±0.04^{aA}	9.66±0.13^{bcB}	6.94±0.06^{eB}	**8.49±0.04^{fA}**	1.08±0.01^{dB}	2.35±0.04^{cA}	**2.04±0.02^{fA}**	1.81±0.02^{deB}	1.78±0.02^{dA}	1.49±0.05^{efB}	76.73±0.04^{cA}	76.21±0.10^{bcB}
Adyang adyang	10.71±0.15^{abA}	8.98±0.03^{cB}	7.25±0.01^{deB}	9.02±0.10^{cA}	**1.04±0.01^{fB}**	2.47±0.08^{bA}	2.23±0.08^{dA}	1.75±0.03^{eB}	2.06±0.04^{bA}	1.76±0.01^{bB}	76.70±0.14^{cA}	76.21±0.21^{cB}
Total mean	10.00±0.14^{cA}	8.69±0.08^{dB}	7.47±0.03^{cdB}	9.20±0.06^{cA}	1.14±0.01^{cdB}	2.37±0.05^{cA}	2.56±0.12^{bc}	2.11±0.03^{cd}	2.00±0.03^{c}	1.60±0.02^{d}	76.85±0.77^{bc}	76.01±0.66^{cd}

Values reported as mean ± standard deviation. Means down the same column containing different superscript small letters are significantly different and indicate differences among millet varieties. Means in the same row bearing different superscript capital letters differ significantly ($p < 0.05$) and indicate differences between flours from non-germinated and germinated millet within the same millet variety.

4.1.2 Protein

Flour from germinated millet contained the highest protein content in the Odyera variety (9.97%), followed by the *Ekaama* variety (9.59%) and the *Ekwangapel* variety (9.44%). Comparably, protein content was high in *Odyera* with 2% spirulina (12.15%), followed by *Ekwangapel* with 2% spirulina (9.58%) and *Ekaama* with 2% spirulina (9.62%), while the highest protein content was observed in *Odyera* fortified with 10% spirulina (15.76%). Overall, flour from germinated millet showed an increase in protein content from 7.47% in flour from non-germinated millet to 9.32% in germinated millet, indicating that germination increased ($p < 0.05$) protein content by 23.1% (Table 9).

Based on the Recommended Dietary Allowance (RDA) of 13–19 g/day of protein for children aged 6 to 59 months, 100 g of flour from germinated millet provides approximately 49 to 72% of the daily protein requirement, compared to 39 to 57% contributed by flour from non-germinated millet. Although germination substantially improves protein content and nutritional quality, the flour alone does not supply 100% of the RDA for protein, emphasizing the need for further fortification or dietary diversification to achieve complete protein adequacy in children's complementary diets. High protein content is a desirable attribute for flours intended for complementary foods for children aged 6 to 59 months, as it supports growth, muscle and tissue development, enzyme and hormone synthesis, and immune function, thereby promoting healthy physical and cognitive development (Ojo *et al*., 2017).

The increase in protein content during germination is primarily due to enzymatic activity that activates proteases, which hydrolyze complex storage proteins into simpler and more digestible amino acids and peptides, thereby improving protein accessibility and digestibility (Dhliwayo, Chopera, Matsungo, Chidewe, Mukanganyama, Nyakudya and Nyanga, 2023; Sruthi and Rao, 2021). Additionally, the reduction in carbohydrate mass during germination increases the relative proportion of protein per 100 g dry weight, while mobilization of stored nitrogen for amino acid synthesis in the growing embryo further elevates protein levels (Dhliwayo *et al*., 2023). The protein content values obtained in this study were within the recommended limits of the Uganda Standard (min. protein content, 6.8%) (US EAS 89:2017), indicating that the flours partially meet the nutritional quality requirements for millet-based complementary foods. The mean protein content values (9.44–12.15%) obtained in this study are in agreement to those reported by Kasule *et al*. (2023) for finger millet cultivated in Uganda, which contained protein levels ranging from 7–12%.

4.1.3 Fat

Flour from non-germinated millet had the highest fat content in the *Odyera* variety (1.27%) and the lowest in *Adyang adyang* (1.04%). Similarly, flour from germinated millet had the highest fat content in the *Ayuku manyige* variety (2.61%) and the lowest in *Emoro moru* (2.03%). Generally, flour from germinated millet showed an increase in fat content from 1.27% in non-germinated millet to 2.58% in germinated millet, indicating that germination increased ($p < 0.05$) fat content by 107.9% (Table 9). High fat content is a desirable attribute for flours intended for complementary foods for children aged 6 to 59 months, as it enhances energy density, improves mouthfeel, and aids in the absorption of fat-soluble vitamins (A, D, E, and K). The relatively high fat content observed in germinated millet flour in this study suggests that the millet varieties used contain appreciable amounts of lipids beneficial for child growth and development. Similar findings were reported by Ojo *et al*. (2017), who noted that germination increases lipid availability through enzymatic breakdown of complex reserves, thereby improving the nutritional quality of cereal flours. However, excessive fat levels may predispose the flour to oxidative rancidity during storage, which can affect flavour and reduce shelf-life. Therefore, maintaining moderate fat content is important to balance nutritional benefits and product stability.

The increase in fat content during germination is primarily due to enzymatic activity that activates lipases, which hydrolyze triglycerides into free fatty acids, thereby improving fat. Additionally, the reduction in carbohydrate mass during germination increases the relative proportion of fat per 100 g dry weight. The fat content values obtained in this study were within the recommended limits of the Uganda Standard (max. fat content, 2.0%) (US EAS 89:2017). The mean fat content values (1.27–2.58%) obtained in this study are in agreement to those reported by Kasule *et al*. (2023) for flour from germinated millet, which contained fat levels ranging from 2.0–5.0%. Similarly, Sharma (2023) also reported that fat content in millet flour ranged from 1.31 to 6.25%. However, the fat contents of flour germinated millet in this study were higher than the maximum fat content of 2.0% specified in the Uganda Standards (US EAS 89:2017).

4.1.4 Ash

Total ash content reflects the overall mineral composition of the flour samples. Flour from non-germinated millet had the highest ash content in the *Ekaama* variety (3.67%) and the lowest in *Luk caa* (2.04%). Similarly, flour from germinated millet had the highest ash content in the

Ekaama variety (2.87%) and the lowest in *Emoro moru* (1.68%). Generally, flour from germinated millet showed a decrease in ash content from 3.67% in non-germinated millet to 2.87% in germinated millet, indicating that millet flour is rich in micronutrients and has the potential to improve nutrient density when used in composite flour formulations, and that germination significantly decreased ash content by 17.6% ($p < 0.05$) (Table 9).

The decrease in ash content during germination is primarily due to utilization of the available minerals during germination and assimilation of mineral elements into the cell components (Obadina, Ishola, Adekoya, Soares, de Carvalho and Barboza, 2016). The ash content values obtained in this study were within the recommended limits of the Uganda Standard (max. ash content, 4.2%) (US EAS 89:2017). The mean ash content values (3.67–2.87%) obtained in this study are in agreement to those reported by Kumar *et al*. (2021) for millet flour from, which contained fat levels ranging from 3.25 to 4.99%. Variation in ash content among the flours can be attributed to inherent differences in botanical composition and mineral density of the raw materials. The superior ash content in millet flour strongly supports its nutritional advantage in fortifying millet flour, particularly where micro-nutrient deficiencies are a public health concern. Its incorporation can enhance the dietary mineral intake of consumers, contributing to improved health outcomes.

4.1.5 Fiber

Flour from non-germinated millet had the highest fiber content in the *Ekaama* variety (2.35%) and the lowest in *Min bel* (1.64%). Similarly, flour from germinated millet had the highest fiber content in the *Ekaama* variety (1.88%) and the lowest in *Min bel* (1.28%). Generally, flour from germinated millet showed a decrease in fiber content from 2.35% in non-germinated millet to 1.88% in germinated millet. indicating that germination decreased ($p < 0.05$) fiber content by 20.0% (Table 9).

The low fiber content in flour from germinated millet is likely due to the enzymatic breakdown of fiber components like hemicellulose and cellulose during germination, as the seed mobilizes nutrients for growth (Dhliwayo *et al*., 2023). Additionally, germination could be due to breakdown of polysaccharides into more soluble sugars which reduced the fiber content (Sharma and Anurag, 2023). The fiber content values obtained in this study were within the recommended limits of the Uganda Standard (max. fiber content, 3.0%) (US EAS 89:2017). The mean fiber content values (2.35 to 1.88%) obtained in this study are in agreement to those reported by Abioye *et al*. (2022) for millet flour from, which contained fiber levels ranging from 1.32 to 2.95%.

4.1.6 Carbohydrate

Flour from non-germinated millet had the highest carbohydrate content in the *Emoro moru* variety (80.12%) and the lowest in *Odyera* (75.43%). Similarly, flour from germinated millet had the highest carbohydrate content in the *Emoro moru* variety (79.02%) and the lowest in *Odyera* (73.85%). Generally, flour from germinated millet showed a decrease in carbohydrate content from 80.12% in non-germinated millet to 79.02% in germinated millet. indicating that germination decreased ($p < 0.05$) carbohydrate content by 1.1%**,** (Table 9). This confirm the role of carbohyfrate as a primary energy source. High starch levels promote good gelatinization and crumb structure in baked products, essential for soft and elastic doughs.

The low carbohydrate content in flour from germinated millet is likely due to the enzymatic breakdown of complex carbohydrates into simple sugars to fuel seed growth during germination (Dhliwayo *et al*., 2023). The mean carbohydrate content values (80.12–79.02%) obtained in this study are in agreement to those reported by Abioye *et al*. (2022) for millet flour from, which contained carbohydrate levels ranging from 65.0 to 83.3%.

4.2 Mineral concentration

Flour from non-germinated millet had the highest calcium content in the *Odyera* variety (329.23 mg/100 g) and the lowest in *Luk caa* (321.50 mg/100 g). Similarly, flour from germinated millet had the highest calcium content in the *Odyera* variety (388.15 mg/100 g) and the lowest in *Luk caa* (348.86 mg/100 g) (Table 10). Generally, flour from germinated millet had increased calcium content (388.15 mg/100 g) compared to flour from non-germinated millet (329.23 mg/100 g), indicating that germination increased ($p < 0.05$) calcium content by 12.7%**,** (Table 9). Based on the Recommended Dietary Allowance (RDA) of 260 to 700 mg/day of calcium for children aged 6 to 59 months, 100 g of flour from germinated millet provides approximately 56 to 149% of the daily calcium requirement, compared to 47 to 127% contributed by flour from non-germinated millet. Millet flours are rich sources of calcium, but children's actual intake is limited due to small porridge portions and varied diets. Germination significantly increases calcium content and enhances nutritional value.

High calcium levels are beneficial for complementary foods for children aged 6 to 59 months. Calcium supports bone and teeth formation, muscle contraction, blood clotting, and nerve function. These qualities make germinated millet flours valuable for promoting healthy growth and development. Although these values indicate that millet flours are rich calcium sources, actual intake is unlikely to cause hypercalcemia because children typically consume smaller portions of flour in porridge form and obtain calcium from a variety of foods. Nonetheless, the results demonstrate that germination significantly enhances the calcium content and potential nutritional contribution of millet-based complementary foods. High calcium content is a desirable attribute for flours intended for complementary foods for children aged 6 to 59 months, as it supports bone and teeth formation, muscle contraction, blood clotting, and nerve function, thereby promoting strong skeletal development and overall growth (Ojo *et al*., 2017).

The increase in calcium content after germination could be attributed to a reduction in phytate and oxalate levels, as these compounds are known to inhibit calcium absorption (Dhliwayo *et al*., 2023). The phytase enzyme break down the phytate complexes, releasing them and making them more extractable and measurable. The mean calcium content values (329.23 to 388.15 mg/100 g) obtained in this study are comparable to those reported by Hassan *et al*. (2021), who found calcium contents ranging from 162 to 487 mg/100 g in millet flours. Similarly, Abioye *et al*. (2022) reported calcium content in millet ranging between 159.7 and 364.6 mg/100 g.

Table 10: Mineral contents in flour from non-germinated and germinated millet

Millet varieties	Calcium (mg/100 g)		Magnesium (mg/100 g)		Iron (mg/100 g)		Zinc (mg/100 g)	
	Non-germinated	**Germinated**	**Non-germinated**	**Germinated**	**Non-germinated**	**Germinated**	**Non-germinated**	**Germinated**
Ekwangapel	327.61±0.18^{aB}	374.10±0.64^{bcA}	143.75±0.01^{abA}	129.10±0.55^{bB}	7.15±0.02^{bB}	11.49±0.03^{bA}	2.24±0.03^{bB}	4.11±0.01^{abA}
Ekaama	324.70±0.07^{aB}	372.70±0.90^{cA}	133.93±0.40^{cdA}	120.89±0.14^{eB}	5.11±0.02^{eB}	9.42±0.01^{dA}	1.91±0.03^{deB}	3.81±0.01^{bcA}
Emoro-moru	327.08±0.09^{aB}	374.68±0.49^{bA}	137.78±0.70^{bA}	124.35±0.62^{cB}	6.55±0.05^{cB}	10.98±0.01^{bcA}	2.06±0.01^{cB}	3.94±0.02^{bA}
Ayuku manyige	324.16±0.05^{aB}	365.17±0.60^{dA}	132.17±0.48^{dA}	123.70±0.16^{dB}	**4.73±0.02**^{fB}	**8.55±0.07**^{eA}	1.90±0.03^{eB}	3.64±0.01^{dA}
Luk caa	**321.50±0.10**^{aB}	**348.86±0.74**^{fA}	135.51±0.46^{cA}	126.03±0.54^{bcB}	5.68±0.02^{deB}	9.96±0.01^{cdA}	2.02±0.01^{cdB}	3.74±0.01^{cA}
Odyera	**329.23±0.29**^{aB}	**388.15±0.58**^{aA}	**154.65±0.74**^{aA}	**135.33±0.55**^{aB}	**8.07±0.09**^{aB}	**12.23±0.53**^{aA}	**2.44±0.02**^{aB}	**4.26±0.01**^{aA}
Min bel	321.91±0.06^{aB}	349.47±0.77^{efA}	132.01±0.89^{deA}	122.05±0.36^{deB}	5.97±0.05^{dB}	10.30±0.01^{cA}	**1.90±0.01**^{eB}	**3.62±0.01**^{eA}
Adyang Adyang	324.28±0.20^{aB}	357.34±0.28^{eA}	**129.23±0.12**^{eA}	**117.88±0.61**^{fB}	5.03±0.04^{eB}	9.31±0.07^{deA}	1.98±0.03^{dB}	3.66±0.02^{dA}
Total mean	325.06±0.13^{aB}	366.31±0.63^{dA}	137.38±0.44^{cA}	124.92±0.48^{bB}	6.04±0.04^{cdB}	10.28±0.09^{cA}	2.06±0.02^{cB}	4.30±0.01^{aA}

Values reported as mean ± standard deviation. Means down the same column containing different superscript small letters are significantly different and indicate differences among millet varieties. Means in the same row bearing different superscript capital letters differ significantly ($p < 0.05$) and indicate differences between flours non-germinated and germinated within the same millet variety.

Flour from non-germinated millet had the highest magnesium content in the *Odyera* variety (154.65 mg/100 g) and the lowest in *Adyang adyang* (129.23 mg/100 g). Similarly, flour from germinated millet had the highest magnesium content in the *Odyera* variety (135.33 mg/100 g) and the lowest in *Adyang adyang* (117.88 mg/100 g). Generally, flour from germinated millet had decreased magnesium content (135.33 mg/100 g) compared to flour from non-germinated millet (154.65 mg/100 g), indicating that germination decreased ($p < 0.05$) magnesium content by 9.1%**,** (Table 10). Based on the RDA of 75 to 130 mg/day for children aged 6 to 59 months, 100 g of flour from germinated millet provides 104 to 180% of the daily magnesium requirement, compared to 119 to 206% from flour from non-germinated millet. Actual intake is lower since children consume small amounts of porridge, and absorption is affected by diet. Germination slightly reduces magnesium, but the flour remains a rich source. High magnesium supports bone development, energy production, muscle and nerve function, and regulates blood glucose and pressure. Adequate intake also enhances calcium and vitamin D metabolism and promotes growth and immunity in children (Ojo *et al*., 2017).

The decrease in magnesium content after germination could be attributed to its utilization as a vital nutrient for the developing seedling's growth and metabolic activities. This reduction may also result from the breakdown and dilution of storage compounds within the seed to provide energy, as well as possible leaching losses during soaking and germination (Dhliwayo *et al*., 2023).The mean magnesium content values (154.65 to 135.33 mg/100 g) obtained in this study are comparable to those reported by Hassan *et al*. (2021), who found magnesium contents ranging from 84.71 to 567.45 mg/100 g in millet flours.

Flour from non-germinated millet had the highest iron content in the *Odyera* variety (8.07 mg/100 g) and the lowest in *Ayuku manyige* (4.73 mg/100 g). Similarly, flour from germinated millet had the highest iron content in the *Odyera* variety (12.23 mg/100 g) and the lowest in *Ayuku manyige* (8.55 mg/100 g) (Table 10). Generally, flours from germinated millet had increased iron content (12.23 mg/100 g) compared to flour from non-germinated millet (8.07 mg/100 g), indicating that germination increased ($p < 0.05$) iron content by 70.2%**,** (Table 10). Based on the Recommended Dietary Allowance (RDA) of 7–11 mg/day of iron for children aged 6 to 59 months, 100 g of flour from germinated millet provides approximately 111–175% of the daily iron requirement, compared to 73–115% contributed by non-germinated millet flour. Although these values exceed 100% of the RDA, the actual iron intake is lower in practice since children consume smaller portions of flour when prepared as porridge, and iron bioavailability from plant sources is limited by antinutritional factors such as phytates. Nevertheless, the findings indicate that germination significantly enhances the iron content and potential nutritional value of millet-based complementary foods.

High iron content is a desirable attribute for flours intended for complementary foods for children aged 6 to 59 months, as iron is essential for the formation of hemoglobin and myoglobin, which facilitate oxygen transport in the blood and muscles (Medise, 2021).. Adequate iron intake supports brain development, cognitive function, and immune system strength, while preventing iron deficiency anemia a common cause of stunted growth, fatigue, and delayed psychomotor development in young children (Ojo *et al*., 2017).

The increase iron content after germination could be due to the activation of phytase enzymes that hydrolyzes the link between iron and phytate (phytic acid) **(**Sruthi and Rao, 2021). The mean iron content values (8.07 – 12.23 mg/100 g) obtained in this study are comparable to those reported by Sharma and Anurag (2023), who found iron contents ranging from 5.98 and 12.39 mg/100g in flours from millet non-germinated pearl millet. Similarly, he found iron content between 12.39 to 40.12 mg/100 g in flours from germinated finger millet. This implies that the findings of iron content in flour from germinated millet were within the reported range.

Flour from non-germinated millet had the highest zinc content in the *Odyera* variety (2.44 mg/100 g) and the lowest in *Min bel* (1.90 mg/100 g). Similarly, flour from germinated millet had the highest zinc content in the *Odyera* variety (4.26 mg/100 g) and the lowest in *Min bel* (3.62 mg/100 g) (Table 10). Generally, flour from germinated millet had increased zinc content (4.26 mg/100 g) compared to flour from non-germinated millet (2.44 mg/100 g), indicating that germination increased ($p < 0.05$) zinc content by 108.7%**,** (Table 10). Based on the Recommended Dietary Allowance (RDA) of 3–5 mg/day of zinc for children aged 6–59 months, 100 g of flour from germinated millet provides approximately 85–142% of the daily zinc requirement, compared to 49–81% contributed by flour from non-germinated millet. Although these values appear high, actual zinc intake is lower in practical feeding situations since children consume smaller portions of flour when prepared as porridge, and zinc absorption from plant-based foods is limited by phytates and other antinutritional factors. These results demonstrate that germination significantly improves the zinc content and enhances the potential contribution of millet-based complementary foods to children’s zinc intake.

High zinc content is a desirable attribute for flour intended for complementary foods for children aged 6 to 59 months, as zinc is crucial for growth, cell division, immune function, and wound healing. Adequate zinc intake supports normal physical development, enhances appetite, strengthens resistance to infections, and aids in the proper functioning of enzymes and hormones involved in metabolism. Deficiency in zinc can lead to growth retardation, weakened immunity, and delayed wound healing in children (Ojo *et al*., 2017).

The increase zinc content after germination could be due to the activation of phytase enzymes that hydrolyzes the link between zinc and phytate (phytic acid) **(**Sruthi and Rao, 2021). The mean zinc content values (2.44 – 4.26 mg/100 g) obtained in this study are comparable to those reported by Ramashia (2021), who found zinc contents ranging from 4.04 – 4.80 mg/100 g in raw millet flours. Similarly, Shobana *et al*. (2013) found zinc content between 0.7 and 3.7 mg/100 g in millet flour. This implies that the findings of zinc content in flours from germinated millet were within the reported range.

4.3 Anti-nutritional factors present in millet

Flour from non-germinated millet had the highest oxalate content in the *Odyera* variety (19.97 mg/100 g) and the lowest in *Ekwangapel* (11.79 mg/100 g). Similarly, flour from germinated millet had the highest oxalate content in the *Odyera* variety (5.20 mg/100 g) and the lowest in *Luk caa* (1.90 mg/100 g) (Table 11). Generally, flour from germinated millet had decreased oxalate content (2.77 mg/100 g) compared to flour from non-germinated millet (15.40 mg/100 g), indicating that germination significantly reduced oxalate levels by approximately 82.0% ($p < 0.05$). Low oxalate content is a desirable attribute for flours used in complementary foods for children aged 6 to 59 months, as it enhances the bioavailability of key minerals such as calcium, iron, and zinc. Oxalates can bind to essential minerals such as calcium, iron, and zinc to form insoluble complexes that reduce their bioavailability, leading to nutrient deficiencies. In addition, excessive oxalate intake can contribute to the formation of kidney stones by combining with calcium to form calcium oxalate crystals in the urinary tract. Therefore, the reduction of oxalate content through germination is nutritionally beneficial, as it minimizes these harmful effects while enhancing the utilization of vital minerals by the body (Dhliwayo *et al*., 2023; Awolu *et al*., 2017).

The decrease oxalate content after germination could be due to leaching of soluble oxalates into the soaking water and activation of oxalate oxidase enzymes that breakdown oxalates into carbon dioxide and formate which are less harmful (Dhliwayo *et al*., 2023) The mean oxalate content values (15.40 – 2.77 mg/100 g) obtained in this study are in agreement to those reported by Goudar *et al*. (2023), who found oxalate contents ranging from 3.41 – 39.38 mg/100g in flours from non-germinated millet. Similarly, Abioye *et al*. (2022) found oxalate content between 0.39 and 1.48 mg/100 g in flours from germinated millet. This implies that the findings of oxalate content in germinated millet flours were within the reported range.

Table 11: Levels of Anti-nutritional factors in flour from non-germinated and germinated millet

Millet varieties	**Oxalates (mg/100g)**		**Phytates (mg/100g)**		**Tannins (mg/100g)**	
	Non-germinated	**Germinated**	**Non-germinated**	**Germinated**	**Non-germinated**	**Germinated**
Ekwangapel	**11.79±0.24**^{fA}	3.11±0.19^{bB}	**11.28±0.38**^{eA}	9.82±0.21^{cB}	**1.84±0.01**^{aA}	0.68±0.04^{abB}
Ekaama	14.12±0.55^{eA}	2.85±0.26^{cB}	14.67±0.57^{cA}	9.86±0.42^{cB}	1.32±0.02^{dA}	0.47±0.04^{cdB}
Emoro-moru	10.69±0.46^{gA}	2.58±0.30^{cB}	14.23±0.52^{cdA}	**13.11±0.34**^{aB}	1.39±0.05^{bcA}	**0.72±0.02**^{aB}
Ayuku manyige	14.71±0.29^{eA}	2.16±0.15^{dB}	15.92±0.35^{bA}	11.49±0.56^{bB}	1.54±0.06^{bA}	0.57±0.02^{bB}
Luk caa	18.66±0.61^{bA}	**1.90±0.41**^{efB}	**21.37±0.93**^{aA}	**7.63±0.53**^{fB}	1.37±0.05^{cA}	0.49±0.02^{cB}
Odyera	**19.97±0.63**^{aA}	**5.20±0.14**^{aB}	14.62±1.01^{cA}	11.25±0.51^{bcB}	1.80±0.04^{abA}	0.56±0.03^{bB}
Min bel	16.15±0.88^{dA}	2.36±0.38^{cdB}	13.49±0.89^{dA}	8.86±0.25^{dB}	**0.80±0.03**^{fA}	**0.45±0.04**^{dB}
Adyang adyang	17.14±0.62^{cA}	1.96±0.22^{eB}	13.31±1.50^{deA}	7.88±0.41^{eB}	0.99±0.03^{eA}	0.47±0.04^{cdB}
Total mean	15.40±0.0.54^{eA}	2.77±0.26^{bcB}	14.86±0.77^{bcA}	9.99±0.40^{cB}	1.38±0.04^{cA}	0.55±0.03^{bcB}

Values reported as mean ± standard deviation. Means down the same column containing different superscript small letters are significantly different and indicate differences among millet varieties. Means in the same row bearing different superscript capital letters differ significantly ($p < 0.05$) and indicate differences between flour from non-germinated and germinated millet within the same millet variety.

Flour from non-germinated millet had the highest phytate content in the *Luk caa* variety (21.37 mg/100 g) and the lowest in *Ekwangapel* (11.28 mg/100 g). Similarly, flour from germinated millet had the highest phytate content in the *Emoro moru* variety (13.11 mg/100 g) and the lowest in *Luk caa* (7.63 mg/100 g) (Table 11). Generally, flour from germinated millet had decreased phytate content (9.99 mg/100 g) compared to flour from non-germinated millet (14.86 mg/100 g), indicating that germination significantly reduced phytate levels by approximately 32.8% ($p < 0.05$). Low phytate content is a desirable attribute for flours used in complementary foods for children aged 6 to 59 months, as it improves the bioavailability of essential minerals such as iron, zinc, calcium, and magnesium. Phytates (inositol hexakisphosphate) are known to chelate these minerals, forming insoluble complexes that limit their absorption in the gastrointestinal tract, which can lead to micronutrient deficiencies. High phytate intake has been associated with impaired growth, weakened immunity, and poor bone mineralization in children. The reduction of phytate content during germination enhances mineral utilization, supports healthy growth, strengthens immunity, and improves overall nutritional quality of the millet flour (Eliot *et al.*, 2016; Dhliwayo *et al.*, 2023).

The decrease phytate content after germination phytate content could be due to the activation of phytase enzyme, which hydrolyzes phytates into lower inositol phosphates that release bound minerals (Dhliwayo *et al.*, 2023) The mean phytate content values (14.86 – 9.99 mg/100g) obtained in this study are in agreement to those reported by Sharma *et al*. (2023), who found phytate contents ranging from 15.11 – 8.34 mg/100 g in finger millet. This implies that the findings of phytate content in germinated millet flours were within the reported range.

Flour from non-germinated millet had the highest tannin content in the *Ekwangapel* variety (1.84 mg/100 g) and the lowest in *Min bel* (0.80 mg/100g). Similarly, flour from germinated millet had the highest tannin content in the *Emoro moru* variety (0.72 mg/100 g) and the lowest in *Min bel* (0.45 mg/100 g) (Table 11). Generally, flours from germinated millet had decreased tannin content (0.55 mg/100 g) compared to non-germinated flours (1.38 mg/100 g), indicating that germination significantly reduced tannin levels by approximately 60.1% ($p < 0.05$). Low tannin content is a desirable attribute for flours used in complementary foods for children aged 6 to 59 months, as it enhances protein digestibility and the bioavailability of essential amino acids and minerals such as iron and zinc. Tannins are polyphenolic compounds that can bind to dietary proteins and digestive enzymes, forming insoluble complexes that reduce protein digestibility and nutrient absorption. High tannin intake has been associated with reduced growth performance, poor protein utilization, and lower iron absorption, which may contribute to anemia and malnutrition in children. The reduction of tannin content during germination minimizes these negative effects, thereby improving protein quality, enhancing iron and zinc absorption, and increasing the overall nutritional value of the millet flour (Dhliwayo *et al*., 2023; Eliot *et al*., 2016).

The decrease in tannin content after germination could be due to the activation of oxidative enzymes such as polyphenol oxidase and tannase, which degrade complex tannin molecules into simpler, less astringent phenolic compounds. Additionally, leaching of water-soluble tannins into the soaking medium during germination may further contribute to the reduction. These processes lower the antinutritional effects of tannins, thereby improving protein digestibility and mineral bioavailability in the germinated millet flour (Dhliwayo *et al*., 2023). The mean tannin content values (1.38 – 0.55 mg/100g) obtained in this study are in agreement to those reported by Sharma *et al*. (2023), who found tannin contents ranging from 0.72 – 0.45 mg/100g in flour from germinated millet. Similarly, Abioye *et al*., (2022), who found tannin contents ranging from 2.23 – 1.20 mg/100g in flour from germinated millet. This implies that the findings of tannin content in flours from germinated millet were within the reported range.

4.4 Protein, iron and β-carotene content of millet spirulina composite flours

Spirulina–millet composite flour recorded the highest protein content in *Odyera* fortified with 10% spirulina (15.76%) and the lowest in *Ekwangapel* with 2% spirulina (9.58%). Specifically, *Odyera* with 2% spirulina had a protein content of 12.15% (Table 12), providing approximately 68–94% of the Recommended Dietary Allowance (RDA) per 100 g of flour. In comparison, *Ekaama* with 2% spirulina (9.62%) and *Ekwangapel* with 2% spirulina (9.58%) contributed about 53–74% of the RDA, respectively. These findings demonstrate that spirulina fortification significantly enhances the protein density of millet-based flours, allowing them to meet a substantial portion of the daily protein requirements of infants and young children when incorporated into complementary diets.

The observed increase in protein content in spirulina–millet composite flours is primarily attributed to the inclusion of spirulina, which contains 60–70% high-quality, easily digestible protein rich in essential amino acids (Alfadhly *et al*., 2022). For instance, Odyera fortified with 10% spirulina showed the highest protein content (15.76%), while the 2% spirulina formulations of *Odyera, Ekaama,* and *Ekwangapel* contained 12.15%, 9.62%, and 9.58%, respectively. The enhancement in protein levels results from the dense and bioavailable protein matrix in spirulina, which complements the amino acid composition of germinated millet, thereby improving both protein quality and digestibility. Consequently, these composite flours are highly suitable for addressing the protein needs of children aged 6 to 59 months.

The protein content values obtained in this study (9.58–15.76%) fall within the recommended limits of the Uganda Standard (minimum protein content of 6.8%) (US EAS 89:2017), confirming that the developed flours meet the nutritional quality requirements for millet-based complementary foods. These values are in agreement to those reported by Koli *et al*. (2022) for spirulina-enriched pasta, which contained protein levels ranging from 11.52% to 20.44%. The time it takes for the nutritional effects of spirulina–millet composite flour to manifest in malnourished children depends on the severity of malnutrition and the consistency of dietary intake. Generally, noticeable improvements in weight gain, muscle tone, and energy levels may occur within 4–8 weeks of regular consumption, especially when the flour is incorporated daily into the child's complementary diet. Biochemical indicators such as serum protein or hemoglobin levels may show significant improvement after 8–12 weeks of continuous feeding. However, full nutritional recovery requires a sustained balanced diet that includes other essential nutrients, proper hygiene, and healthcare support to ensure optimal absorption and utilization of nutrients.

Table 12: Protein, iron and beta-carotene content in spirulina millet composite flours

Spirulina	Protein (%)			Iron (mg/100g)			Beta carotene (mg/kg)		
Proportions (%)	***Ekaama***	***Ekwangapel***	***Odyera***	***Ekaama***	***Ekwangapel***	***Odyera***	***Ekaama***	***Ekwangapel***	***Odyera***
2	9.62 ± 0.01^{kB}	**9.58 ± 0.01^{lC}**	12.15 ± 0.01^{eA}	**10.32 ± 0.00^{kB}**	10.34 ± 0.00^{jB}	13.13 ± 0.00^{efA}	**68.12 ± 0.37^{hA}**	68.13 ± 2.30^{hA}	71.13 ± 5.91^{gA}
4	9.94 ± 0.01^{jkC}	9.99 ± 0.01^{jB}	13.05 ± 0.01^{dA}	11.22 ± 0.00^{iC}	12.14 ± 0.01^{hB}	14.03 ± 0.00^{dA}	142.93 ± 1.04^{fA}	141.90 ± 3.83^{fgA}	156.26 ± 23.07^{eA}
6	10.11 ± 0.03^{iB}	10.16 ± 0.06^{hB}	13.95 ± 0.01^{cA}	12.19 ± 0.01^{gC}	13.05 ± 0.01^{fB}	14.93 ± 0.02^{cA}	215.59 ± 1.13^{deA}	216.59 ± 2.57^{dA}	228.93 ± 22.02^{cdA}
8	11.31 ± 0.01^{fgB}	10.65 ± 0.01^{gC}	14.86 ± 0.01^{bA}	13.02 ± 0.00^{fgC}	13.97 ± 0.01^{eB}	15.83 ± 0.00^{bA}	284.78 ± 0.94^{bcA}	279.45 ± 5.92^{cA}	288.11 ± 5.76^{bA}
10	11.75 ± 0.02^{fB}	11.71 ± 0.06^{fB}	**15.76 ± 0.02^{aA}**	13.93 ± 0.01^{eC}	14.87 ± 0.01^{cdB}	**16.73 ± 0.01^{aA}**	349.56 ± 7.71^{abA}	352.89 ± 6.54^{aA}	**353.24 ± 11.48^{aA}**

Values are reported as mean ± standard deviation. Superscripts indicate significant differences ($p < 0.05$): **lowercase letters down columns** show differences among varieties, and **uppercase letters across** rows show differences between spirulina levels

Spirulina–millet composite flour contained the highest iron content in *Odyera* with 10% spirulina (16.73 mg/100 g) and the lowest in *Ekaama* with 2% spirulina (10.32 mg/100 g). Specifically, *Odyera* with 2% spirulina had an iron content of 13.13 mg/100 g (Table 12), providing approximately 131–188% of the RDA per 100 g of flour, while *Ekaama* 2% spirulina (10.32 mg/100 g) and *Ekwangapel* 2% spirulina (10.34 mg/100 g) contributed about 103–147% and 103–148%, respectively. Overall, these results demonstrate that spirulina fortification markedly enhances the iron density of millet-based flours, enabling them to meet and even exceed the daily iron requirements of infants and young children when appropriately portioned and incorporated into complementary diets.

Although the calculated RDA contribution appears to exceed 100%, this does not necessarily pose a risk of iron overload or toxicity. The actual amount of flour consumed by children per meal is typically less than 100 g, and the bioavailability of non-heme iron from plant-based **foods** such as millet and spirulina is relatively low, estimated at 5–10% due to the presence of residual phytates, fiber, and polyphenols (Hurrell and Egli, 2010). Moreover, iron absorption is regulated physiologically according to the body's needs, which minimizes the likelihood of hyperaccumulation. Therefore, the high iron density observed in spirulina–millet composite flours is nutritionally advantageous, particularly in addressing iron-deficiency anemia among children in low-income settings.

The increase in iron content observed in spirulina–millet composite flours is primarily attributed to the inclusion of spirulina powder, which is exceptionally rich in bioavailable iron compared to millet and other cereal ingredients (Morsy, 2014). For instance, *Odyera* fortified with 10%

spirulina recorded the highest iron content (16.73 mg/100 g), while *Odyera* 2% spirulina, *Ekaama* 2% spirulina, and *Ekwangapel* 2% spirulina flours contained 13.13 mg/100 g, 10.32 mg/100 g, and 10.34 mg/100 g (Table 12), respectively. The substantial enhancement in iron content is due to the high intrinsic iron concentration in spirulina, coupled with its low phytic acid content, which minimizes mineral binding and enhances iron bioavailability (Saharan and Jood, 2017). This fortification effect is further supported by the fact that spirulina iron is largely present in chelated forms, which are more easily absorbed than the non-heme iron typically found in cereals. Consequently, increasing spirulina supplementation proportionally elevates the total and bioavailable iron content of the composite flours, making them valuable for improving iron intake and combating iron deficiency anemia among children aged 6 to 59 months. The mean iron content values (10.32–16.73 mg/100 g) obtained in this study are consistent with those reported by Zuluaga *et al*. (2021) for spirulina-fortified cereal blends, which ranged from 9.8 to 18.4 mg/100 g, confirming the nutritional enhancement potential of spirulina fortification in complementary food formulations.

Spirulina–millet composite flour contained the highest beta-carotene content in *Odyera* with 10% spirulina (353.24 mg/ kg) and the lowest in *Ekaama* with 2% spirulina (68.12 mg/kg). Specifically, *Odyera* with 2% spirulina had beta-carotene iron content of 71.13 mg/ kg, followed by *Ekwangapel* of 68.13 mg/ kg and followed by 68.12 mg/ kg (Table 12). Overall, these results demonstrate that spirulina fortification markedly enhances the β-carotene density of millet-based flours, thereby improving their provitamin A potential and contributing to better vision, immune function, and overall growth in infants and young children. The increase in β-carotene content observed in spirulina–millet composite flours is primarily attributed to the incorporation of spirulina, which is naturally rich in carotenoid pigments such as β-carotene, zeaxanthin, and cryptoxanthin that serve as potent provitamin A sources (Soni *et al*., 2021). For instance, *Odyera* fortified with 10% spirulina exhibited the highest β-carotene content, followed by *Odyera* 2% spirulina, *Ekaama* 2% spirulina, and *Ekwangapel* 2% spirulina flours, indicating that increasing the level of spirulina supplementation proportionally enhances β-carotene concentration. The improvement in β-carotene levels is further linked to the high stability of carotenoids in spirulina and their lipid-soluble nature, which facilitates better absorption and conversion to vitamin A in the human body (Beheshtipour et al., 2013). This enhancement not only improves the provitamin A potential of the flours but also contributes to the prevention of vitamin A deficiency, supports normal vision, boosts immune function, and promotes healthy growth and development in children aged 6–59 months. The β-carotene levels obtained in this study align with those reported by Kamel *et al*. (2020), who observed increased carotenoid density in cereal–spirulina blends, underscoring the nutritional significance of spirulina fortification in improving the vitamin A value of complementary foods.

The mean β-carotene content values obtained in this study were consistent with those reported by previous researchers for spirulina-fortified cereal products. Saharan and Jood (2017) reported β-carotene concentrations ranging from 2.31 to 7.81 mg/100 g in composite blends, while Sathya et al. (2014) found much lower levels, below 0.01 mg/100 g in plain millet flour and between 0.46 and 2.83 µg/g (equivalent to 0.046–0.283 mg/100 g) in millet grains. In contrast, spirulina powder contains substantially higher β-carotene concentrations, reported between 700 and 1700 mg/kg (Chaouachi *et al*., 2024) and 150–250 mg/100 g (equivalent to 1500–2500 mg/kg) by AlFadhly *et al.* (2022). These findings confirm that the incorporation of spirulina significantly enhances the β-carotene density of millet-based composite flours, thereby improving their provitamin A value and demonstrating the strong nutritional enrichment potential of spirulina fortification in complementary food formulations.

4.5 Sensory acceptability of the spirulina millet based composite product (porridge)

Appearance: Each of the three millet-based flours (*Ekaama, Ekwangapel,* and *Odyera*) had control samples (100% millet) that generally scored highly for most sensory attributes. Germination was observed to improve all sensory qualities, resulting in better appearance, color, taste, and overall acceptability across the flours. The incorporation of spirulina powder into millet flour produced a characteristic vibrant green hue, which influenced the visual appearance of the porridge. Among the formulations, *Odyera* composite flour with 2% spirulina powder received the highest mean appearance score (7.90), whereas *Odyera* with 10% spirulina recorded the lowest score (4.53) (Table 13). The appearance of the of the porridge varied for each composite flour ($P < 0.05$). The distinct appearance of porridges prepared from spirulina–millet composite flours could be attributed to the natural pigments, particularly chlorophyll and carotenoids, present in spirulina, which imparted a greenish coloration to the product. This observation is consistent with findings from previous studies, where the inclusion of spirulina or other green biomass ingredients enhanced the color intensity of food products (Shyampriya *et al*., 2019; Beheshtipour *et al*., 2013).

C**olor**, there was significant difference ($p < 0.05$) between the mean ratings of each formulation. Color scores tended to decline with higher spirulina levels, as observed in *Odyera* with 10% spirulina, which received lower ratings (4.07) compared to *Odyera* with 2% spirulina (7.77)

(Table 13). Overall, moderate spirulina fortification (2%) maintained consumer acceptability and visual appeal comparable to the germinated millet control, whereas higher inclusion levels negatively affected color perception and appearance scores. These findings indicate that incorporating spirulina at lower concentrations can enhance the nutritional quality of millet-based porridges without compromising sensory acceptability, thereby supporting its potential use in formulating acceptable and nutrient-enriched complementary foods for children.

To enhance the acceptability of spirulina-fortified products based on their color sensory attributes, several measures can be applied. Incorporating natural ingredients such as milk powder, vanilla, or fruit extracts like banana or mango can soften the intense green coloration and create a more appealing visual tone while improving the overall aroma and flavor of the product. Maintaining a moderate spirulina inclusion level of about 1–2% helps to balance nutritional enhancement with an acceptable color and sensory appeal. Blending spirulina with lighter-colored flours such as rice, maize, or sorghum before mixing with millet can further dilute the deep green hue, producing a lighter and more visually acceptable product. Additionally, educating caregivers and consumers about the nutritional benefits of the natural green color, mainly derived from chlorophyll and carotenoids, can improve their psychological acceptance of the product’s appearance. Finally, enhancing packaging and labeling to emphasize the “natural green, nutrient-rich” identity of the flour can positively influence consumer perception and acceptance, encouraging wider adoption of spirulina-based complementary foods.

Flavour: The mean flavour scores of porridges prepared from millet-based composite flours containing 8% and 10% spirulina were lower. The results indicated that increase in spirulina proportion reduces the flavor of the formulations. The flavour of the of the porridge varied for each composite flour ($P < 0.05$). The reduction in flavour acceptability with increasing spirulina concentration may be attributed to the intensified green color and the distinct taste imparted by spirulina, which together diminish the overall palatability of the porridge. Among the formulations, *Ekaama* composite flour with 2% spirulina recorded the highest mean flavour score (7.13), while *Odyera* with 10% spirulina had the lowest (5.07) (Table 13). The progressive decline in flavour scores with higher spirulina inclusion levels indicates that spirulina's strong umami and marine-like taste can overpower the mild flavour profile of millet when used in large proportions. Overall, moderate spirulina fortification (2%) preserved the desirable flavour characteristics and consumer acceptability of the porridge, whereas higher levels (8–10%) negatively influenced flavour perception. These results suggest that low-level spirulina enrichment can be effectively used to improve the nutritional value of millet-based complementary foods without compromising flavour acceptability.

Table 13: Sensory scores of the porridge containing different proportions of spirulina millet composite flours

Attribute	Variety	Spirulina percentages						
		NG-0%	**G-0%**	**2%**	**4%**	**6%**	**8%**	**10%**
Appearance	***Odyera***	6.67±1.69^{bC}	7.87±0.82^{aA}	**7.90±0.76^{aA}**	5.67±1.14^{cE}	6.27±0.96^{bcD}	4.83±1.18^{deG}	**4.53±1.11^{deG}**
	Ekaama	6.73±1.14^{abB}	7.77±0.82^{aA}	7.17±1.37^{abB}	6.93±1.17^{abB}	6.27±1.02^{bcD}	5.53±1.41^{cE}	4.80±0.76^{dF}
	Ekwangapel	6.57±1.28^{bC}	7.33±1.24^{aA}	6.40±0.89^{bcD}	6.57±1.25^{bC}	6.27±1.20^{bcD}	5.53±1.57^{cE}	4.80±1.58^{dF}
Colour	***Odyera***	6.40±1.43^{abB}	7.87±0.94^{aA}	**7.77±.82^{aA}**	5.30±0.79^{bcD}	6.20±1.45^{bC}	4.43±1.07^{cE}	**4.07±0.74^{dG}**
	Ekaama	6.40±0.93^{bC}	7.63±0.67^{aA}	7.33±1.21^{aA}	6.67±0.61^{abB}	5.53±0.86^{bcD}	4.53±1.11^{cE}	4.23±0.82^{cdF}
	Ekwangapel	6.80±1.56^{aA}	7.20±1.13^{aA}	6.67±1.03^{aA}	7.17±1.56^{aA}	5.53±1.25^{bC}	4.53±1.38^{cE}	4.23±1.43^{cdF}
Flavour	***Odyera***	7.03±0.67^{abB}	7.63±0.96^{aA}	7.03±0.85^{abB}	6.17±1.15^{bcD}	6.50±0.90^{bC}	5.73±0.79^{dF}	**5.07±1.20^{deG}**
	Ekaama	6.43±0.94^{bC}	7.67±0.84^{aA}	**7.13±0.73^{abB}**	7.10±0.77^{abB}	6.80±0.71^{bC}	6.03±0.81^{cE}	5.47±1.04^{dF}
	Ekwangapel	6.80±0.85^{bC}	7.33±0.99^{aA}	7.07±0.88^{abB}	6.97±1.54^{bC}	6.80±1.19^{bC}	6.03±1.33^{cE}	5.47±1.63^{dF}
Texture	***Odyera***	6.73±0.82^{cD}	7.67±106^{abB}	6.93±0.83^{cD}	**8.10±0.99^{aA}**	7.10±1.42^{bC}	**6.03±1.69^{dE}**	6.67±1.45^{cD}
	Ekaama	6.93±0.69^{cD}	7.70±0.70^{abB}	7.40±1.13^{bC}	8.00±0.64^{aA}	8.10±0.66^{aA}	7.97±0.67^{abB}	6.73±0.74^{cD}
	Ekwangapel	6.70±0.84^{cD}	7.20±0.81$_{bC}$	7.07±0.94^{cD}	7.27±1.48^{bC}	8.07±1.11^{aA}	7.97±0.67^{abB}	6.73±0.69^{cD}

Values are mean scores ± standard deviation from 90 panelists; SP: Spirulina powder; G: Germinated; NG: Non Germinated; values with different superscript in the same column are significantly ($p<0.05$) different. Control sample are made from 100% germinated millet flour.

Table 14: Sensory scores of the porridge containing different proportions of spirulina millet composite flours

Attribute	**Variety**	**Spirulina percentages**						
		NG-0%	**G-0%**	**2%**	**4%**	**6%**	**8%**	**10%**
Mouthfeel	***Odyera***	6.93±0.69^{abB}	7.83±0.99^{aA}	6.93±0.79$_{abB}$	7.37±1.00^{aA}	6.27±1.98^{bC}	**5.87±1.53^{cD}**	6.37±1.40^{abB}
	Ekaama	6.23±0.43^{bC}	6.90±0.71^{abB}	6.23±0.86$_{bC}$	**8.13±0.63^{aA}**	7.37±1.43^{aA}	7.97±0.67^{aA}	6.73±0.74^{abB}
	Ekwangapel	6.97±0.85^{abB}	7.40±0.81^{aA}	6.73±0.63^{abB}	6.70±1.34^{abB}	7.37±1.13^{aA}	7.97±0.62^{aA}	6.73±1.51^{abB}
Taste	***Odyera***	6.87±1.55^{aA}	7.57±1.01^{aA}	**8.03±0.89^{aA}**	5.07±0.98^{cdE}	6.07±1.31^{bcC}	4.87±1.04dF	3.77±1.25^{eG}
	Ekaama	7.07±1.51^{aA}	7.50±0.90^{aA}	7.10±1.47^{aA}	6.73±0.87^{bB}	5.53±0.82^{cD}	5.10±1.16^{cD}	**3.37±0.93^{eG}**
	Ekwangapel	8.03±0.62^{aA}	7.13±1.48^{aA}	6.47±1.07^{bB}	6.83±1.32^{bB}	5.53±0.90^{cD}	5.10±1.35^{cD}	3.37±1.13^{eG}
General acceptability	***Odyera***	7.37±0.77^{abB}	7.97±0.89^{aA}	7.23±1.04^{bC}	6.57±1.04^{bcD}	6.27±0.83^{cE}	5.40±0.93^{cdF}	**5.20±1.00^{dG}**
	Ekaama	7.67±0.88a^{bB}	8.20±0.71^{aA}	**7.57±0.82^{abB}**	7.47±0.73^{abB}	6.67±0.76^{bcD}	5.97±0.85^{cE}	5.30±0.95^{cdF}
	Ekwangapel	7.23±0.68^{bC}	7.50±0.82^{aA}	6.67±1.27^{bcD}	6.63±1.38^{bcD}	6.57±1.36^{bcD}	5.97±1.47^{cE}	5.30±1.92^{cdF}

Values are mean scores ± standard deviation from 90 panelists; SP: Spirulina powder; G: Germinated; NG: Non Germinated; values with different superscript in the same column are significantly ($p<0.05$) different. Control sample are made from 100% germinated millet flour.

Mouthfeel: The mean mouthfeel scores of porridges prepared from spirulina–millet composite flours varied with spirulina concentration. *Ekaama* composite flour with 4% spirulina received the highest mean mouthfeel score (8.13), while *Odyera* with 8% spirulina recorded the lowest score (5.87) (Table 13). The mouthfeel of the of the porridge varied for each composite flour ($P < 0.05$). The decline in mouthfeel acceptability at higher spirulina levels may be due to the increased density and slightly fibrous texture imparted by spirulina powder, which can affect the smoothness and overall palatability of the porridge. Overall, moderate spirulina inclusion (2–4%) maintained a smooth and acceptable mouthfeel comparable to the germinated millet controls, whereas higher levels (8–10%) negatively influenced textural perception. These findings suggest that low to moderate spirulina fortification can improve the nutritional quality of millet-based porridges without compromising mouthfeel, making them suitable for consumption by children aged 6–59 months.

Texture: The mean texture scores of porridges prepared from spirulina–millet composite flours differed significantly with spirulina concentration. *Odyera* composite flour with 4% spirulina obtained the highest mean texture score (8.10), while *Odyera* with 8% spirulina recorded the lowest score (6.03) (Table 13). The texture of the porridge varied significantly among the formulations ($P < 0.05$). The decrease in texture acceptability at higher spirulina levels may be attributed to the increased viscosity and coarse granularity introduced by spirulina powder, which can reduce the smooth and cohesive consistency typical of well-gelled millet porridge. In contrast, moderate spirulina inclusion (2–4%) retained a desirable soft and uniform texture similar to that of the germinated millet controls. These results indicate that low to moderate levels of spirulina fortification enhance the nutritional profile of millet-based porridges while maintaining acceptable texture characteristics suitable for complementary feeding of children aged 6–59 months.

Taste: The mean taste scores of porridges prepared from spirulina–millet composite flours varied significantly with spirulina concentration. *Odyera* composite flour with 2% spirulina recorded the highest mean taste score (8.03), while *Ekaama* with 10% spirulina had the lowest score (3.37) (Table 13). The taste of the porridges differed significantly among the formulations (P < 0.05). The reduction in taste acceptability at higher spirulina levels could be attributed to the strong earthy and slightly bitter flavor of spirulina, which becomes more pronounced with increasing concentration and may mask the natural sweetness of millet. In contrast, porridges with low spirulina inclusion (2–4%) retained a pleasant, mild, and well-balanced flavor comparable to germinated millet controls. These findings indicate that low levels of spirulina fortification enhance the nutritional composition of millet-based porridges while maintaining acceptable taste characteristics, making them suitable for complementary feeding of children aged 6 to 59 months.

The mean overall acceptability scores of porridges prepared from spirulina–millet composite flours decreased with increasing spirulina concentration. *Ekaama* composite flour with 2% spirulina recorded the highest mean overall acceptability score (7.57), while *Odyera* with 10% spirulina had the lowest score (5.20) (Table 13). The overall acceptability of the porridges varied significantly among the formulations (P < 0.05). The decline in overall acceptability at higher spirulina levels may be due to the increased substitution rate, which alters the product's desirable sensory qualities such as color, taste, and texture. As reported by Aljobair *et al*. (2021), high spirulina concentrations impart a greener color that reduces consumer appeal. Similarly, Koli *et al*. (2022) observed that excessive spirulina supplementation diminishes the sensory attractiveness of food products. In contrast, moderate spirulina inclusion (around 2–4%) was found to enhance both nutritional and sensory properties, consistent with findings by Morsy *et al*. (2014), who reported highest acceptability at 2.5% spirulina, and Muresan *et al*. (2016), who found that 2–5% spirulina fortification improved the nutritional quality and sensory perception of pasta. Overall, the present findings suggest that low spirulina incorporation (approximately 2%) optimizes consumer acceptance while significantly improving the nutritional quality of millet-based porridges for children aged 6 to 59 months.

4.6 Principal Correlation Analysis of Nutritional and Sensory Properties

The PCA biplot (Figure 4) illustrates the relationships among nutritional and sensory attributes of millet–spirulina composite porridges and how these variables differentiate the samples. The two main principal components, F1 and F2, accounted for 84.83% of the total variation, with F1 contributing 67.81% and F2 contributing 17.01%. F1 represented attributes mainly related to sensory quality and nutritional composition, including appearance, color, flavor, taste, overall acceptability, and β-carotene, which had the highest squared cosine values (>0.85). This indicates that samples with positive F1 scores were associated with better sensory appeal and higher carotenoid content, making them more acceptable to consumers.

F2, on the other hand, was dominated by texture (45.41%) and mouthfeel (44.54%), representing the physical or rheological properties of the porridges.

Samples positioned on the right side of the biplot (positive F1 axis) mainly control samples and those with low spirulina inclusion (2 to 4%) were closely associated with desirable sensory traits such as taste, flavor, color, and overall acceptability. This indicates that low spirulina fortification maintained consumer-preferred sensory characteristics while improving nutritional quality. Conversely, samples positioned on the left side of F1 (such as *Odyera*–4%SP and *Odyera*–6%SP) were associated with higher protein and iron content but lower sensory scores, implying a trade-off between nutritional enhancement and palatability at higher spirulina concentrations. Samples like *Ekaama*–2%SP and *Ekaama*–4%SP showed high F2 values, indicating a strong correlation with texture and mouthfeel, suggesting that these formulations achieved a desirable balance between smoothness and viscosity. Meanwhile, samples with negative F2 values had poor mouthfeel or coarse texture, which could negatively affect consumer acceptability.

The PCA results indicate that sensory attributes (F1) and physical texture properties (F2) are the key determinants of consumer acceptability for millet–spirulina porridges. Products positioned in the positive F1 region (controls and 2 to 4% spirulina samples) are most likely to be preferred by consumers, as they combine nutritional enrichment with acceptable sensory quality. Samples with negative F1 and F2 values, though nutritionally rich in protein and iron, may require processing modifications (e.g., finer milling of spirulina, blending with flavor enhancers, or improved gelatinization) to reduce bitterness and coarseness. From a processing perspective, the findings suggest that maintaining spirulina inclusion at low to moderate levels (2 to 4%) is optimal for achieving consumer-acceptable porridges. High levels (≥8%) increase nutrient density but compromise taste, color, and texture. Nutritionally, the PCA highlights that spirulina fortification enhances β-carotene, protein, and iron, but these improvements must be balanced with sensory quality to ensure consumer compliance, especially in complementary feeding programs for children aged 6–59 months.

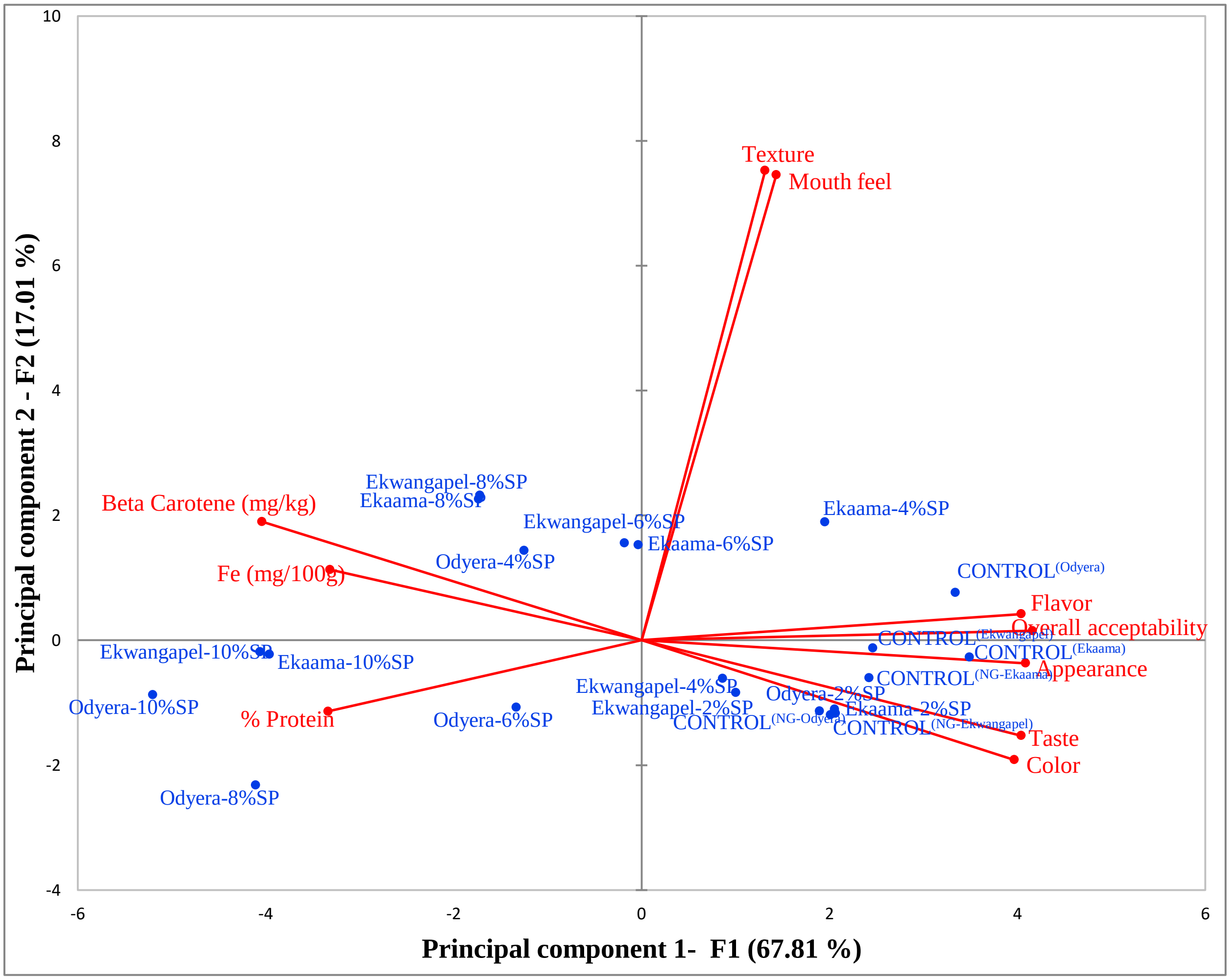

Figure 4: Principal Correlation Analysis of Nutritional and Sensory Properties

Table 15: Showing the principal component analysis:

Eigenvalues:

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Eigenvalue	6.781	1.701	0.947	0.233	0.156	0.067	0.049	0.039	0.019	0.007
Variability (%)	67.812	17.014	9.466	2.335	1.565	0.672	0.487	0.390	0.185	0.074
Cumulative %	67.812	84.826	94.292	96.627	98.192	98.863	99.350	99.740	99.926	100.000

Squared cosines of the variables (PCA):

Attributes	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Appearance	**0.909**	0.002	0.066	0.002	0.001	0.001	0.012	0.001	0.002	0.003
Color	**0.856**	0.050	0.067	0.000	0.007	0.001	0.000	0.011	0.007	0.000
Flavor	**0.888**	0.002	0.050	0.007	0.027	0.007	0.004	0.014	0.001	0.000
Texture	0.094	**0.773**	0.040	0.080	0.009	0.002	0.001	0.001	0.000	0.000
Mouth feel	0.112	**0.758**	0.015	0.114	0.000	0.000	0.000	0.000	0.001	0.000
Taste	**0.889**	0.032	0.031	0.009	0.001	0.029	0.004	0.003	0.002	0.001
Overall acceptability	**0.942**	0.000	0.008	0.001	0.023	0.012	0.011	0.001	0.000	0.002
% Protein	**0.605**	0.018	0.315	0.010	0.048	0.000	0.000	0.002	0.002	0.000
Fe (mg/100g)	**0.598**	0.017	0.348	0.005	0.021	0.005	0.001	0.002	0.003	0.001
β-carotene (mg/kg)	**0.887**	0.049	0.006	0.007	0.018	0.010	0.016	0.005	0.001	0.000

Values in bold correspond for each variable to the factor for which the squared cosine is the largest

4.7 Cosumer preference

The preference mapping (Figure 5) complements the PCA by illustrating how consumer clusters related to different product formulations. Each cluster represents a group of consumers with similar preferences. The preference data (Table 15) revealed that acceptability decreased with increasing spirulina substitution levels. Samples with 10% and 8% spirulina (particularly *Odyera*) were least preferred in clusters 1, 2, and 5, suggesting that strong color and flavor changes reduced consumer liking. In contrast, *Ekaama* with 6% spirulina showed higher preference in clusters 3 and 4, while *Ekaama*–2%SP and control porridges (without spirulina) were most liked across all clusters, especially 1, 2, and 5. This pattern aligns with the PCA findings, where samples with low spirulina inclusion were positioned in regions associated with positive sensory attributes. It further confirms that color, taste, and overall acceptability were the most influential factors guiding consumer preference.

The clustering patterns indicate the existence of distinct consumer segments:

- Clusters 1, 2, and 5 favored porridges with mild flavor and natural millet color, indicating low tolerance for strong spirulina flavor or greenish appearance.
- Clusters 3 and 4 were more open to moderate spirulina inclusion (4 to 6%), appreciating its improved texture and nutritional benefits.

For processing, this means manufacturers should optimize spirulina concentration (2 to 4%) to satisfy the majority of consumers while still achieving nutritional enrichment. For nutrition programs, targeting products in this range can enhance child nutrient intake (protein, iron, β-carotene) without risking rejection due to undesirable sensory attributes.

- **F1 axis (sensory & nutritional attributes)** distinguishes products based on consumer appeal higher values reflect products that are more acceptable and visually attractive.
- **F2 axis (texture & mouthfeel)** differentiates samples by physical feel moderate spirulina inclusion improves smoothness, while excessive inclusion increases coarseness.
- **Clusters from preference mapping** validate that consumers generally prefer products in the **positive F1–F2 region**, corresponding to **2–4% spirulina formulations.**

Thus, PCA and preference mapping together provide evidence that low to moderate spirulina fortification (2 to 4%) achieves an optimal balance between nutrition and sensory quality, guiding future formulation and processing improvements for child-targeted fortified porridges. The outcomes of the Principal Component Analysis provided a clear understanding of how sensory and nutritional parameters collectively influenced the differentiation and acceptability of the millet–spirulina porridges. To further validate these relationships and understand consumer response patterns, a preference mapping analysis was conducted. This approach complements the PCA findings by grouping consumers according to their liking for specific formulations. While the PCA highlighted the correlation between product attributes (such as taste, color, texture, and nutrient content), the preference mapping illustrates how these attributes translated into actual consumer choices and acceptability trends across different clusters.

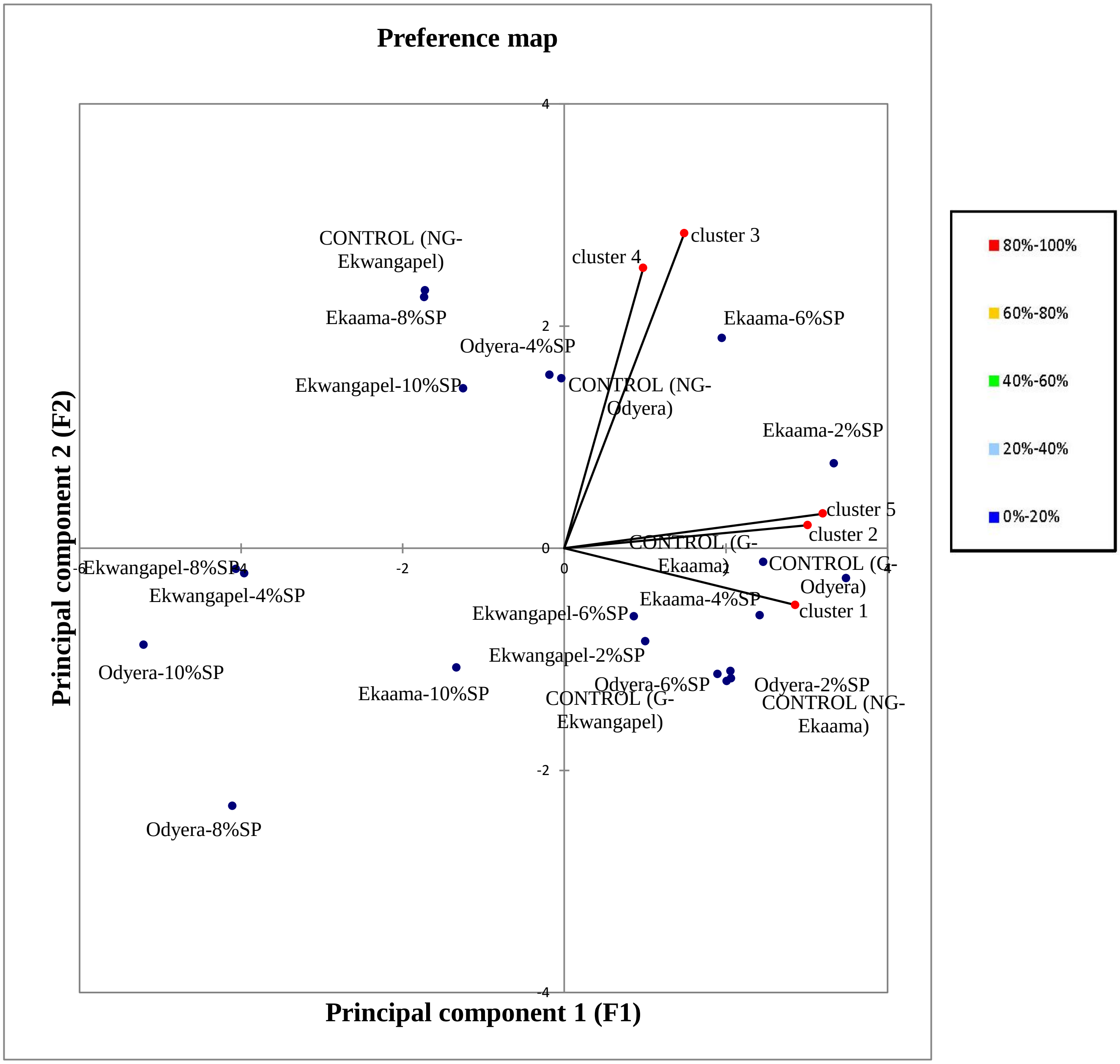

Figure 5: Preference Mapping of millet-spirulina porridge

Table 16: The preference orders for the various groups of consumers

cluster 1	cluster 2	cluster 3	cluster 4	cluster 5
Odyera-10%SP	*Odyera*-10%SP	*Odyera*-8%SP	*Odyera*-8%SP	*Odyera*-10%SP
Ekwangapel-8%SP	*Odyera*-8%SP	*Odyera*-10%SP	*Odyera*-10%SP	*Odyera*-8%SP
Ekwangapel-4%SP	*Ekwangapel*-8%SP	*Ekwangapel*-8%SP	*Ekwangapel*-8%SP	*Ekwangapel*-8%SP
Odyera-8%SP	*Ekwangapel*-4%SP	*Ekwangapel*-4%SP	*Ekwangapel*-4%SP	*Ekwangapel*-4%SP
CONTROL (NG-*Ekwangapel*)	CONTROL (NG-*Ekwangapel*)	*Ekaama*-10%SP	*Ekaama*-10%SP	CONTROL(NG-*Ekwangapel*)
Ekaama-8%SP	*Ekaama*-8%SP	*Ekwangapel*-2%SP	*Ekwangapel*-2%SP	*Ekaama*-8%SP
Ekwangapel-10%SP	*Ekaama*-10%SP	*Ekwangapel*-6%SP	CONTROL (G-*Ekwangapel*)	*Ekaama*-10%SP
Ekaama-10%SP	*Ekwangapel*-10%SP	CONTROL (G-*Ekwangapel*)	*Odyera*-6%SP	*Ekwangapel*-10%SP
Odyera-4%SP	*Odyera*-4%SP	*Odyera*-6%SP	CONTROL (NG-*Ekaama*)	*Odyera*-4%SP
CONTROL (NG-*Odyera*)	CONTROL (NG-*Odyera*)	CONTROL (NG-*Ekaama*)	*Odyera*-2%SP	CONTROL (NG-*Odyera*)
Ekwangapel-6%SP	*Ekwangapel*-6%SP	*Odyera*-2%SP	*Ekwangapel*-6%SP	*Ekwangapel*-6%SP
Ekwangapel-2%SP	*Ekwangapel*-2%SP	*Ekaama*-4%SP	*Ekaama*-4%SP	*Ekwangapel*-2%SP
Ekaama-6%SP	*Odyera*-6%SP	*Ekwangapel*-10%SP	CONTROL (G-*Ekaama*)	*Odyera*-6%SP
Odyera-6%SP	CONTROL (G-*Ekwangapel*)	CONTROL (G-*Ekaama*)	*Ekwangapel*-10%SP	CONTROL (G-*Ekwangapel*)
CONTROL (G-*Ekwangapel*)	*Odyera*-2%SP	CONTROL(NG-*Ekwangapel*)	CONTROL (G-*Odyera*)	*Odyera*-2%SP
Odyera-2%SP	CONTROL (NG-*Ekaama*)	*Ekaama*-8%SP	*Odyera*-4%SP	CONTROL (NG-*Ekaama*)
CONTROL (NG-*Ekaama*)	*Ekaama*-6%SP	*Odyera*-4%SP	CONTROL (NG-*Odyera*)	*Ekaama*-6%SP
CONTROL (G-*Ekaama*)	*Ekaama*-4%SP	CONTROL (NG-*Odyera*)	CONTROL(NG-*Ekwangapel*)	*Ekaama*-4%SP
Ekaama-4%SP	CONTROL (G-*Ekaama*)	CONTROL (G-*Odyera*)	*Ekaama*-8%SP	CONTROL (G-*Ekaama*)
Ekaama-2%SP	*Ekaama*-2%SP	*Ekaama*-2%SP	*Ekaama*-2%SP	*Ekaama*-2%SP
CONTROL (G-*Odyera*)	CONTROL (G-*Odyera*)	*Ekaama*-6%SP	*Ekaama*-6%SP	CONTROL (G-*Odyera*)

CHAPTER FIVE: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

Objective I: Proximate Composition of Germinated and Non-Germinated Millet Flours

Germination significantly improved the nutritional composition of millet across the eight varieties studied. Germination increased ($p < 0.05$) protein content by 23.1%**,** fat by 107.9% and reduced moisture by 13.1%**,** ash by 17.6%**,** fibre by 20.0%**,** carbohydrate by 1.1%**,**

Objective II: Mineral Composition of Germinated and Non-Germinated Millet Flours

Germination increased ($p < 0.05$) calcium by 12.7%**,** iron by 70.2%**,** and zinc by 108.7%**.** and reduced and magnesium by 9.1%**.**

Objective III: Anti-Nutritional Factors of Millet Flours

Germination reduced antinutritional factors as follows, oxalates by **82.0%**, phytates by **32.8%**, and tannins by **60.1%.**

Objective IV: Sensory Acceptability of Spirulina–Millet Composite Porridges

Sensory evaluation revealed that porridge acceptability decreased with increasing spirulina levels. The highest scores for taste (8.03), texture (8.10), mouthfeel (8.13), and overall acceptability (7.57) were obtained from *Ekaama*–2% spirulina, while *Odyera*–10% spirulina recorded the lowest scores for most sensory attributes.

Objective V: Nutritional Composition of Spirulina–Millet Composite Flours

Incorporation of spirulina significantly increased the protein, iron, and β-carotene contents of millet-based composite flours. Protein content increased by up to 25%, iron by 10%, and β-carotene by over threefold compared to germinated millet flour alone.

5.2 Conclusions

Germination increased ($p < 0.05$) protein content by 23.1%**,** fat by 107.9% and reduced moisture by 13.1%**,** ash by 17.6%**,** fibre by 20.0%**,** carbohydrate by 1.1%**.** It increased calcium by 12.7%**,** iron by 70.2%**,** and zinc by 108.7%**.** and reduced and magnesium by 9.1%**.** Germination further decreased antinutritional factors, including oxalates by 82.0%, phytates by 32.8%, and tannins by 60.1%. "Flour from germinated millet is more digestible and nutrient-rich, making it a suitable raw material for complementary food formulations. Incorporating spirulina further increased protein by up to 25%, iron by 10%, and β-carotene more than threefold, producing a nutritionally enriched composite. However, high spirulina levels (above 6%) lowered sensory acceptability because of strong color and flavor changes. The *Ekaama* formulation with 2% spirulina achieved the best sensory scores, demonstrating that low-level spirulina fortification (2 to 4%) maintained desirable sensory attributes while improving micronutrient density. Combining flour from germinated millet with 2 to 4% spirulina produces a nutrient-rich, acceptable porridge for children aged 6 to 59 months. These findings demonstrate the potential for such formulations to contribute to improved dietary diversity and nutrient intake among children in regions vulnerable to micronutrient deficiencies.

5.3 Recommendations

1. To enhance the nutritional value and consumer acceptability of complementary foods, *Ekaama*-based composite flour with 2% spirulina inclusion is recommended for product development. This formulation achieved optimal balance between nutrient enrichment (protein ↑25%, iron ↑60%, β-carotene ↑3×) and sensory appeal.
2. Germination should be adopted as a pre-processing step in the formulation of millet-based composite flours. The germination process improves protein content by up to 18%, enhances mineral concentration, and reduces anti-nutritional factors such as phytates and tannins by 35–75%, thereby improving nutrient bioavailability and digestibility.
3. Although higher spirulina inclusion levels increase micronutrient content, inclusion beyond 4% is not recommended for children's porridge formulations due to reduced sensory acceptability linked to changes in color, flavor, and texture.
4. Further research should focus on determining the bioavailability of protein, iron, and β-carotene in the millet–spirulina composite porridges, as well as stability during storage and cooking to validate their potential contribution to child nutrition.
5. Product optimization studies should also consider cost-effectiveness and large-scale processing feasibility, ensuring that nutrient-rich millet–spirulina blends remain affordable and accessible for households in low-income communities.

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APPENDICES

Appendix 1: Spirulina in its natural form

Figure 5:Picture of spirulina in its natural form (Tadros, 1990)

Appendix 2: Calibration curve for minerals in millet flours

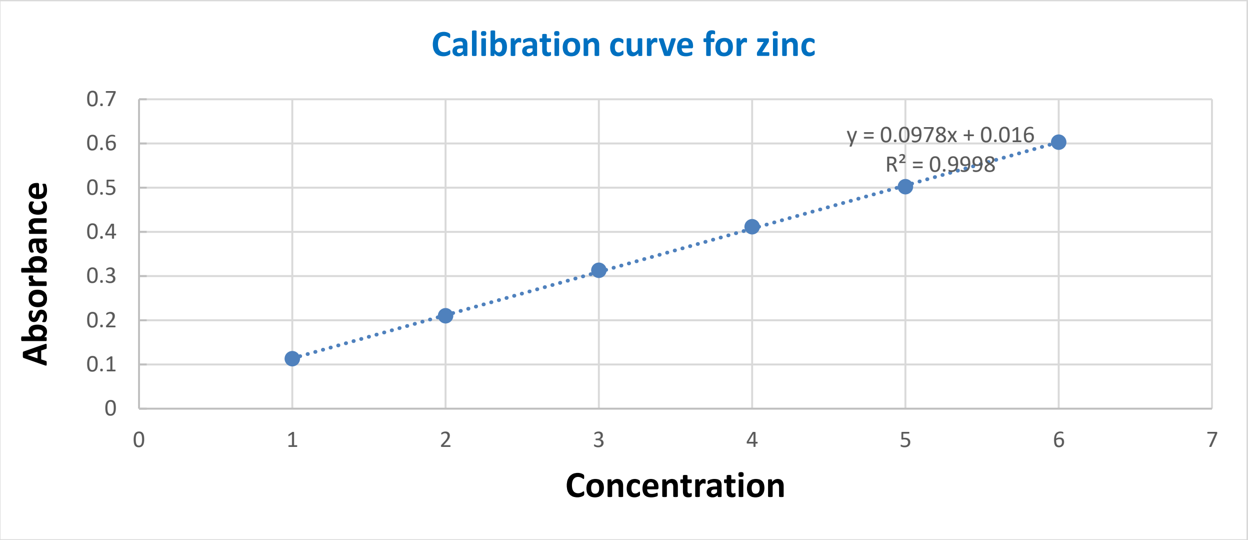

Figure 6: Calibration curve for zinc

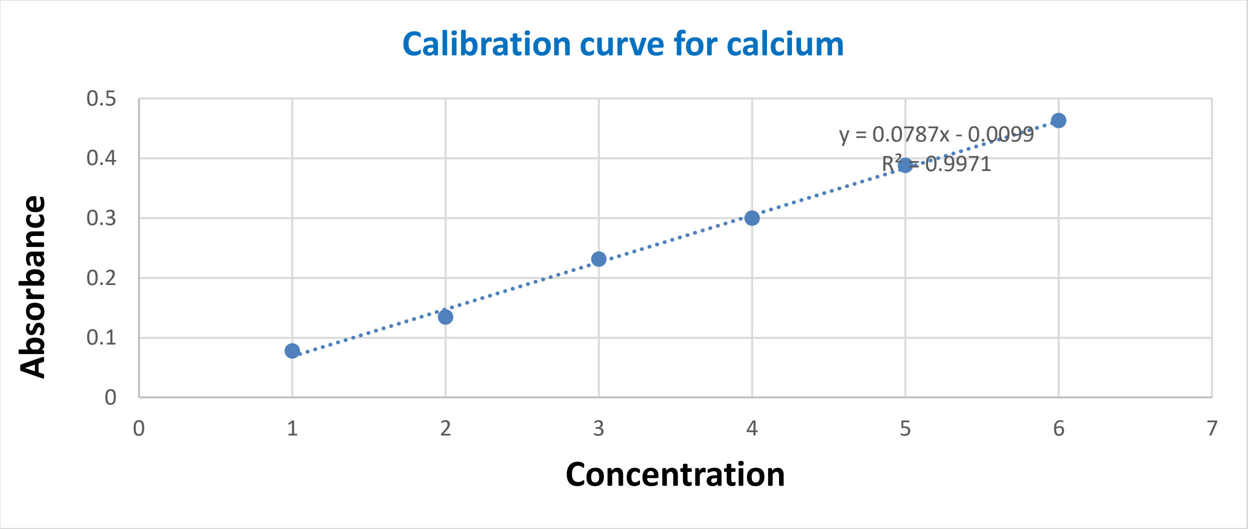

Figure 7: Calibration curve for calcium

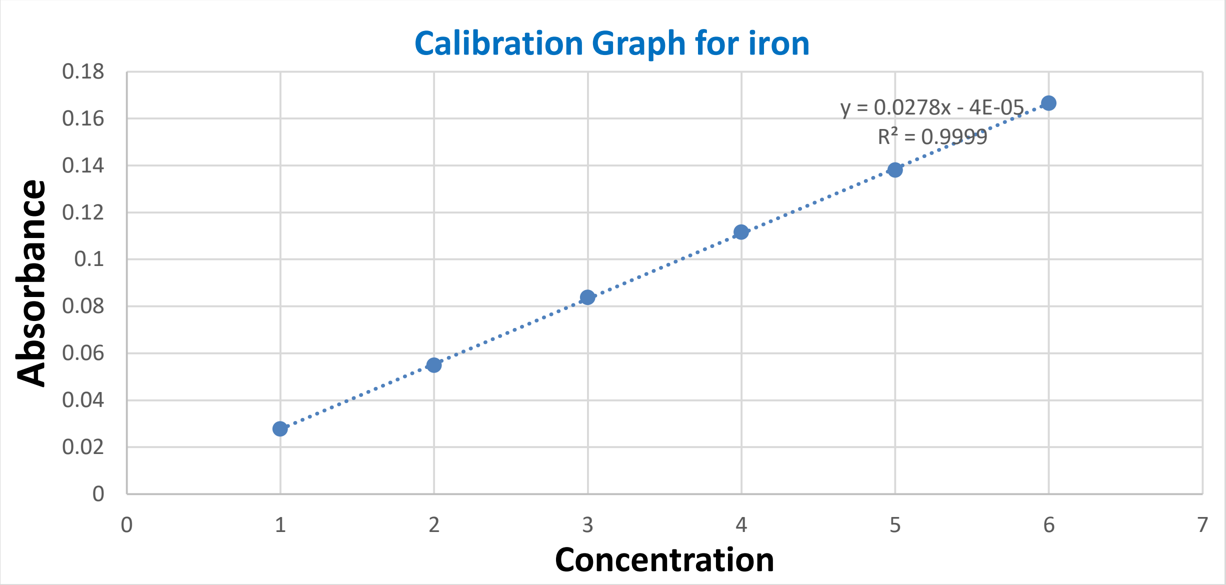

Figure 8: Calibration curve for iron

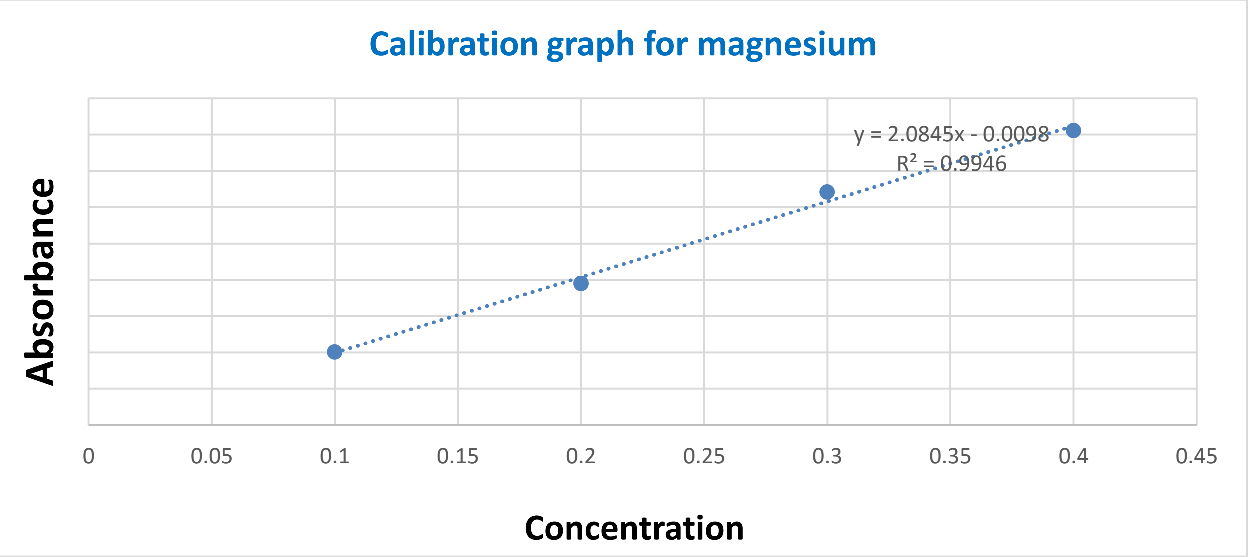

Figure 9: Calibration curve for magnesium

Appendix 3: Calibration curve for Antinutritional factors in the flours

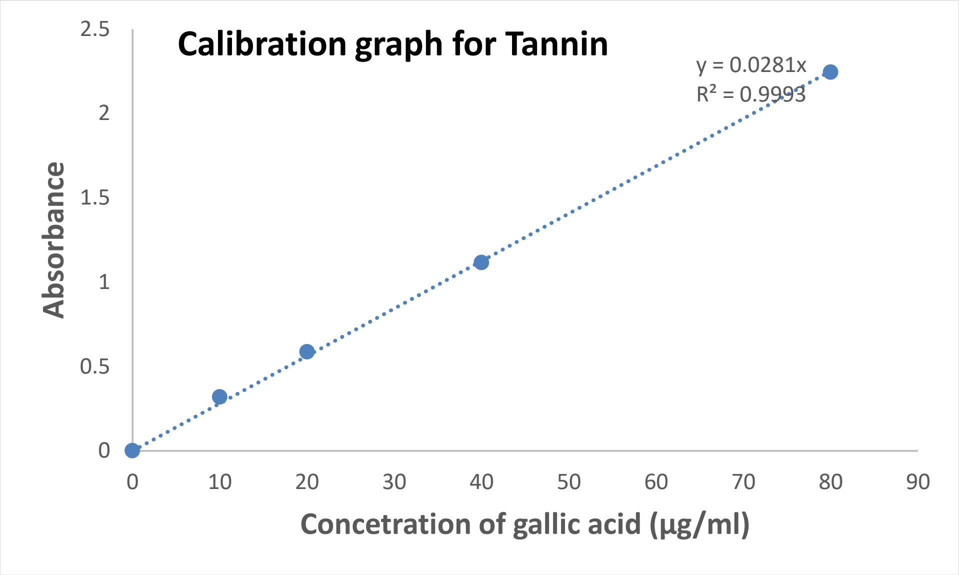

Figure 10: Calibration curve for tannin

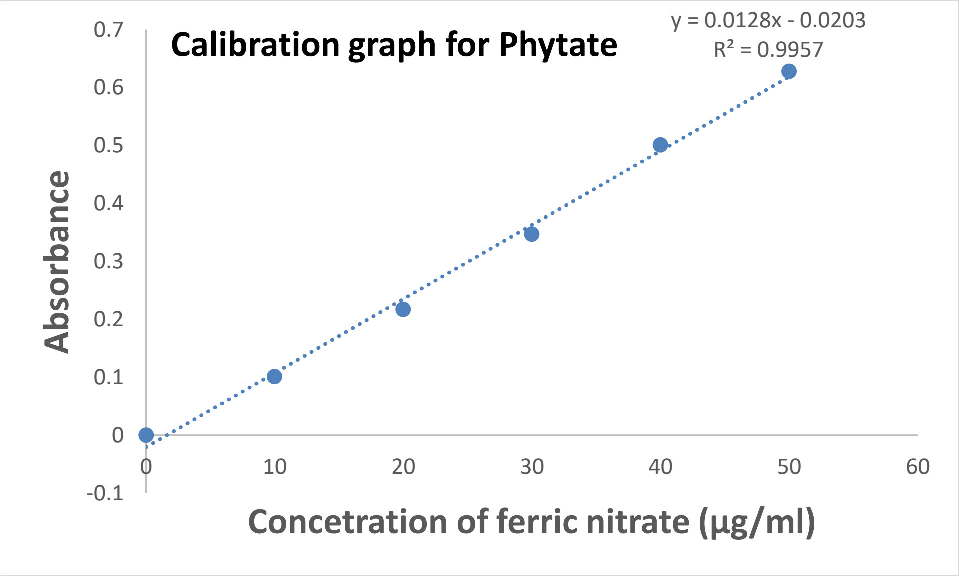

Figure 11: Calibration curve for phytate

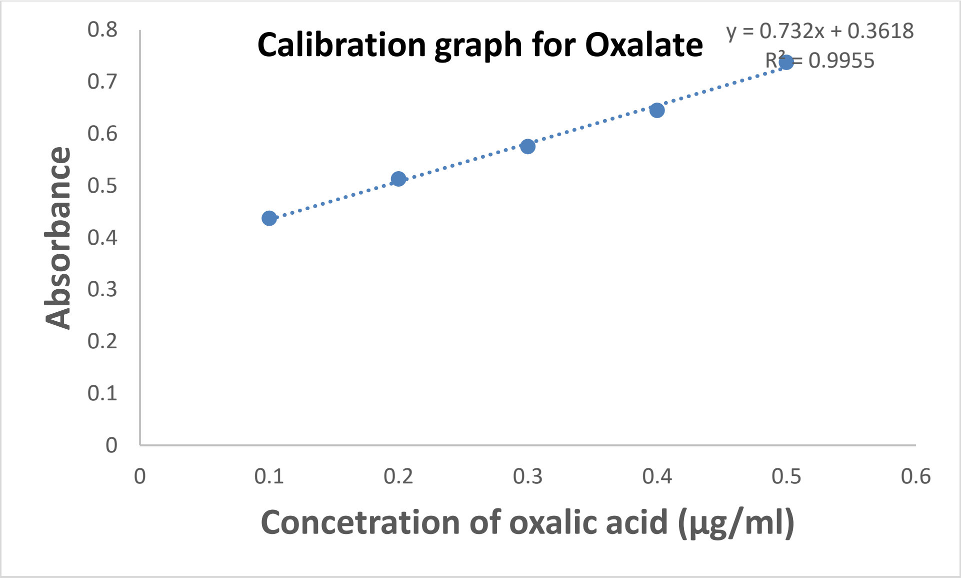

Figure 12: Calibration curve for oxalate

Appendix 4: Consent form

Consent Form for Participation in Research

Study Title:

Nutritional Composition and Sensory Evaluation of Spirulina (Arthrospira platensis) Millet-Based Composite Flour for Children Aged 5–59 Months

Researcher in Charge:

Laker Scovia
Location: Acholi Quarter, Banda B1, Gulu, Kampala, Uganda

Purpose of the Study:

This study aims to:

- Optimize the spirulina-millet composite flour.
- Assess the acceptability of the developed porridge among children under five years old.

Study Procedures:

You are invited to participate as a caregiver/mother who can help evaluate whether children under five like the product. Participation involves:

- A one-time, individual semi-structured interview.
- Duration of about one hour.
- Conducted at a convenient location for the sensory evaluation.
- Taking field notes to ensure accurate recording of responses.

Benefits:

You may not receive direct benefits, but your input will help improve nutritional planning and meal choices for young children.

Risks or Discomforts:

The study involves minimal risk, similar to what you encounter in daily life. No additional risks are expected.

Compensation:

You will receive UGX 5,000 as appreciation for your time.

Privacy and Confidentiality:

Your information will remain private. Only the research team, including the Principal Investigator and those directly involved in the study, may access your records. Any published findings will not include names or identifying information.

Voluntary Participation / Withdrawal:

Participation is entirely voluntary. You may withdraw at any time without penalty or loss of any benefits.

Questions or Concerns:

For questions, concerns, or complaints about the study, contact the researcher at 0779742234. For questions about your rights or issues with the study, contact the Research Supervisor, Dr. Phoebe Kaddumukasa, at 0752616272.

Assessment of Understanding:

Please indicate your understanding of this consent form:

☐ I have read and understood the information and voluntarily agree to participate in the study.

☐ I have read the information but still have questions and do not yet give full consent to participate.

Signature of Person Taking Part in Study: **Date:**

Name of Person Taking Part in Study: **Date:**

Appendix 5: The ballot for sensory evaluation

Panelist No.________________________

You are provided with samples of spirulina millet-based composite porridge prepared with varying contents of millet flours and spirulina powder. Please observe and record your liking of the samples on the scale of 1 to 9 by placing your score in the box next to the sensory parameter under each sample in the table. Please evaluate the products in the order in which they are presented. Use the water provided to refresh your palate before and between samples.

ANSWER ALL QUESTIONS. We would like to know what you think!!

If you have any questions, please ask the study coordinator.

Score the products using hedonic scale below	
Like extremely	**9**
Like very much	**8**
Like moderately	**7**
Like slightly	**6**
Neither like nor dislike	**5**
Dislike slightly	**4**
Dislike moderately	**3**
Dislike very much	**2**
Dislike extremely	**1**

Quality attributes	**Sample No**			
Appearance				
Colour				
Flavour				
Texture				
Mouth feel				

General acceptability				

Which sample (only one) would you most prefer and why?

....…………………………………………...

General comments:

……………………………………………………………………………………………………

Thank you for participating in this exercise

Appendix 6: Ethical Approval from CIU-REC

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RESEARCH ETHICS COMMITTEE

☎ (+256) 0312 307400
✉ rec@ciu.ac.ug
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16/05/2024

To: LAKER SCOVIA

0779742234

Type: Initial Review

Re: CLARKE-2023-836: Antioxidants and Nutritional Potential of Enriched Millet Based Composite Flour Using Spirulina (Arthrospira platensis)

I am pleased to inform you that at the **42nd** convened meeting on **27/02/2024**, the Clarke International University REC meeting voted to approve the above referenced application.
Approval of the research is for the period of **16/05/2024** to **16/05/2025.**

As Principal Investigator of the research, you are responsible for fulfilling the following requirements of approval:

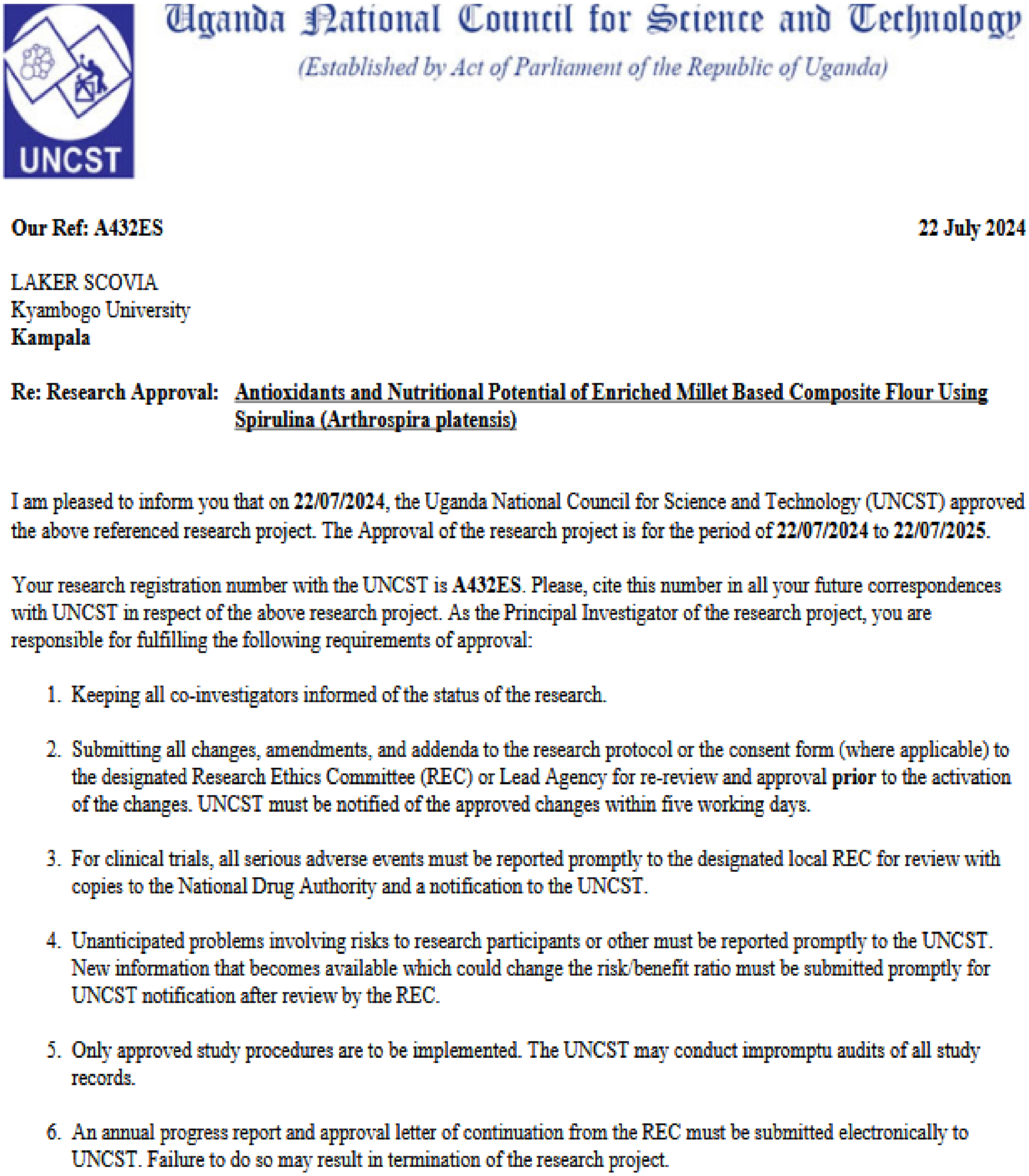
1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the protocol or the consent form must be submitted to the REC for re-review and approval **prior** to the activation of the changes.
3. Reports of unanticipated problems involving risks to participants or any new information which could change the risk benefit: ratio must be submitted to the REC.

No.	Document Title	Language	Version Number	Version Date
1	Protocol	English	2.0	2024-05-15
2	Informed Consent forms	English	01	2024-03-27
3	COVID-19 & EBOLA risk management plan for the study	English	01	2024-03-27
4	Data collection tools	English	01	2024-02-13
5	Community Engagement plan if applicable to your study	English	01	2024-02-13

Yours Sincerely

Samuel Kabwigu
For: Clarke International University REC

Appendix 7: Research permit from UNCST



Uganda National Council for Science and Technology
(Established by Act of Parliament of the Republic of Uganda)
UNCST

Our Ref: A432ES **22 July 2024**

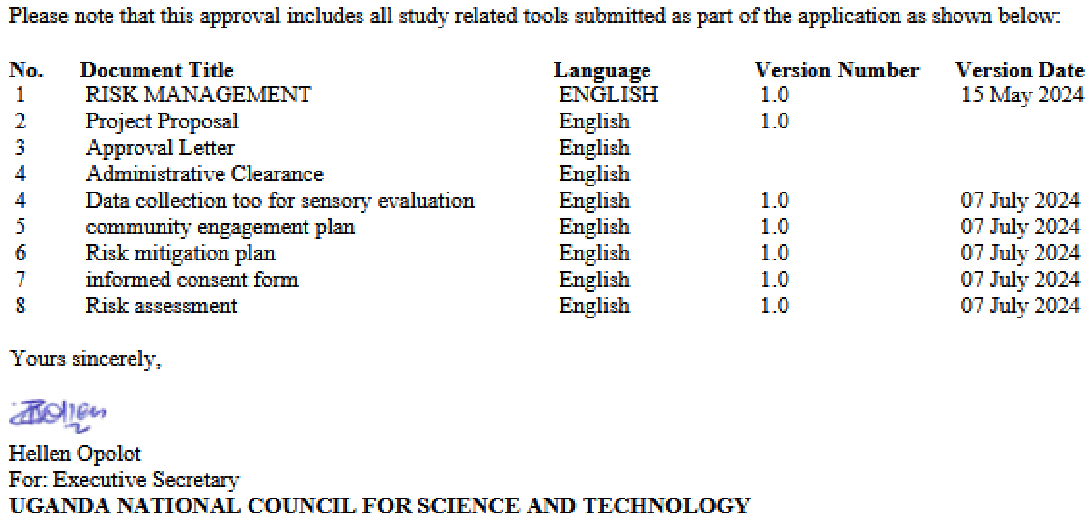
LAKER SCOVIA
Kyambogo University
Kampala

Re: Research Approval: **<u>Antioxidants and Nutritional Potential of Enriched Millet Based Composite Flour Using Spirulina (Arthrospira platensis)</u>**

I am pleased to inform you that on **22/07/2024**, the Uganda National Council for Science and Technology (UNCST) approved the above referenced research project. The Approval of the research project is for the period of **22/07/2024** to **22/07/2025**.

Your research registration number with the UNCST is **A432ES**. Please, cite this number in all your future correspondences with UNCST in respect of the above research project. As the Principal Investigator of the research project, you are responsible for fulfilling the following requirements of approval:

1. Keeping all co-investigators informed of the status of the research.
2. Submitting all changes, amendments, and addenda to the research protocol or the consent form (where applicable) to the designated Research Ethics Committee (REC) or Lead Agency for re-review and approval **prior** to the activation of the changes. UNCST must be notified of the approved changes within five working days.
3. For clinical trials, all serious adverse events must be reported promptly to the designated local REC for review with copies to the National Drug Authority and a notification to the UNCST.
4. Unanticipated problems involving risks to research participants or other must be reported promptly to the UNCST. New information that becomes available which could change the risk/benefit ratio must be submitted promptly for UNCST notification after review by the REC.
5. Only approved study procedures are to be implemented. The UNCST may conduct impromptu audits of all study records.
6. An annual progress report and approval letter of continuation from the REC must be submitted electronically to UNCST. Failure to do so may result in termination of the research project.



Please note that this approval includes all study related tools submitted as part of the application as shown below:

No.	Document Title	Language	Version Number	Version Date
1	RISK MANAGEMENT	ENGLISH	1.0	15 May 2024
2	Project Proposal	English	1.0	
3	Approval Letter	English		
4	Administrative Clearance	English		
4	Data collection too for sensory evaluation	English	1.0	07 July 2024
5	community engagement plan	English	1.0	07 July 2024
6	Risk mitigation plan	English	1.0	07 July 2024
7	informed consent form	English	1.0	07 July 2024
8	Risk assessment	English	1.0	07 July 2024

Yours sincerely,

Hellen Opolot
For: Executive Secretary
UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY